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EFFECT OF HEMICEREBELLECTOMY UPON THE CYTOCHEMISTRY OF NEURONS IN THE LATERAL VESTIBULAR NUCLEUS

I. *Effects on RNA Content and Succinoxidase Activity in Deiters' Neurons at Different Post-operative Intervals*

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Abstract RNA content and succinoxidase activity was measured in single Deiters giant nerve cells from the lateral vestibular nucleus in rabbits subjected to hemicerebellectomy Postoperatively there was a decreased RNA content bilaterally after 1 2 weeks and almost restored values after 6 weeks. The succinoxidase activity showed asymmetrical changes with initially higher values on the operated side and with rise on the contralateral side after 1 week. The succinoxidase activity and RNA content later returned to approximately preoperative values.

The two main components of the vestibular system, i.e. the vestibular nuclei and the cerebellum, are intimately related to each other both structurally and functionally (Brodal et al 1962) The current opinion is that cerebellar structures are stimulated by excitatory impulses coming from the labyrinth organs and the vestibular nuclei (Brodal et al., 1962 Dow 1939), and the resulting output from the cerebellar cortex is inhibitory (Ito & Yoshida, 1964 1966 Jarlstedt, 1966 Obata et al., 1967) In a series of investigations we have studied the complex functional dependence between different parts of the vestibular system using nerve cell RNA and succinoxidase activity on the cellular level (Blomstrand et al 1966 *a*, *b* 1968 *a*, *b* Hallén & Hamberger 1964 Hallén et al., 1967).

After unilateral vestibular neurotomy or repeated unilateral caloric irrigations, we found asymmetrical cytochemical changes in the cerebellum (Blomstrand et al., 1966 *a*, *b*) reflecting its role as an important coordinator of vestibular activity It was also observed that unilateral vestibular neurotomy elevated the succinoxidase activity of Deiters giant cells on the operated side (Blomstrand et al., 1966 *a*) Destruction of the cerebellar nodulus immediately after neurotomy resulted in a disappearance of the former succinoxidase changes (Hallén et al., 1967) which further supports the argument that the vestibular nuclei are functionally dependent on the cerebellum In a recent study we observed significant changes in RNA content and succinoxidase activity in Deiters giant nerve cells after total cerebellectomy (Blomstrand et al., 1968 *a*) Since most of the cerebello-vestibular fibers are uncrossed (Walberg & Jansen, 1961) it was of considerable interest to study the cytochemical balance in the vestibular nuclei after hemicerebellectomy

MATERIAL AND METHODS

White rabbits of both sexes, weighing 1.5–1.8 kg were used The operation was similar to that described earlier for total cerebellectomies

Table I. *RNA content in Deiters giant cells from hemicerebellectomized rabbits (right sided cerebellar ablation) at various postoperative intervals*

Mean values in pg ± S.E.M N.S. = not significant

		Left side		Right side (operated)		
Time after operation	No of animals	No. of cells	Mean value ± S.E.M.	No. of cells	Mean value ± S.E.M.	*P*
1 hour	6	38	1 386 ± 47	37	1 396 ± 38	N.S.
7 days	6	50	1 273 ± 96	49	1 215 ± 84	N.S.
15 days	4	32	946 ± 125	33	911 ± 85	N.S.
40 days	5	43	1 226 ± 110	42	1 253 ± 82	N.S.

(Blomstrand et al., 1968 *a*) with the exception that only the right side of the cerebellum was completely exposed. The vermis was divided sagittally by a pair of scissors This was always possible without much bleeding. After the division the right half of the cerebellum was extirpated by gentle dissection and suction, sparing only a small part of the crura just above the medulla oblongata. When the bleeding stopped, the dura was sutured and covered by gel foam and the muscles and the skin were closed by single nylon monofilament sutures.

ANALYSES

Determination of RNA Content and Succinoxidase Activity

The animals were killed by air emboli after various postoperative intervals. Single Deiters giant nerve cells from the lateral vestibular nucleus were dissected free hand from fresh tissue under a stereomicroscope according to Hydén (Hydén & Pigon 1960)

RNA-determination

The free hand dissected cells were placed on a coverslip and allowed to dry and thereafter fixed in ice-cold 10% perchloric acid for 5 min and subsequently washed in three baths of 0.01 N acetic acid. The coverslips were stored in 95% ethanol at least 24 hours before the determination procedure The RNA content in the single cells was determined according to Edström (1964)

Succinoxidase determination

Succinoxidase activity of isolated nerve cells was determined by measuring the oxygen consumption in an incubation medium containing succinate as a substrate with the use of Zeuthen's microdiver technique (Zeuthen, 1953)

RESULTS

Animals

During the first part of the postoperative period the general condition of the animals deteriorated but after one to two days they began to take water and vegetables The rabbits lost about 400 g during the first week. After this period the original body weight was regained and even surpassed Postoperatively the animals showed dorsal flexion of the neck and rotation to the left. There was often eye deviation to the right, nystagmus to the left and always a tendency of falling to the right and sometimes even for rotation and immediately after operation there was also a bilateral extension of the forelimbs. These symptoms subsided gradually and after 40 days the animals were generally able to sit or to walk around in the cage by leaning against the walls. We found no lesions in the brain stem in the sacrificed animals and histological examination of the remaining part of the cerebellum gave no evidence for degeneration.

RNA

The results are presented in Table I and Fig. 1 The RNA content in isolated Deiters cells markedly decreased during the first part of

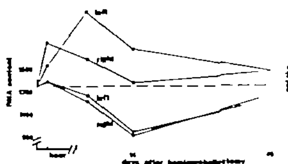


Fig. 1 RNA content (o—o) and succinoxidase activity (●—●) of Deiters giant nerve cells at various time intervals following hemicerebellectomy (right sided ablation). RNA content expressed as pg RNA and enzyme activity as 10^{-4} µl O_2 per hour

the postoperative period and reached a minimum value 15 days postoperatively. The normal value was almost restored after 40 days. At 7 and 15 days the operated side had slightly lower RNA values compared to the unoperated side, but the differences are not statistically significant.

Succinoxidase activity

The results are presented in Table II and in Fig. 1. In contrast to the RNA content, the succinoxidase activity of the Deiters cells showed asymmetrical changes. The Deiters cells on the right side, i.e. ipsilateral to the hemicerebellectomy showed initial high activities. The activity then declined and the values after 15 and 40 days were only slightly higher than in controls. The left side, contralateral to the hemicerebellectomy had an initial succinoxidase activity lower than the right side, a condition which was reversed into higher left side values after 7 days postoperatively. At this day the succinoxidase activity on the left side was 3 times higher than in controls. After the first postoperative week both sides had declining values and after 40 days the respiratory activity was around 30% above the control value.

DISCUSSION

Previous studies demonstrated increased succinoxidase activity in Deiters giant nerve cells after cerebellectomy (Blomstrand et al. 1968 *a*). Based upon the current theory that the Purkinje cell action upon these cells is inhibitory (Ito & Yoshida, 1964, 1966; Jarlstedt, 1966; Obata et al., 1967) the increased enzyme activity was interpretated to be the result of removed cerebellar inhibition. These findings are consistent with the observed values of succinoxidase activity in the Deiters cells 1 hour after hemicerebellectomy. This activity was comparatively higher on the operated side, presumably due to the elimination of the above-mentioned inhibitory influence. At 7 and 15 days postoperatively these conditions were reversed, i.e. higher enzyme activity in the cells contralateral to the operation compared with the operated side, but after 40 days the values were identical and almost at control level.

It is known from a number of studies (v. Eyck, 1956; Flurr, 1961; Blomstrand et al., 1966 a, 1968; Hallén et al., 1967, among others) that injuries to a part of the vestibular

Table II. *Succinoxidase activity in Deiters giant cells from hemicerebellectomized rabbits (right sided cerebellar ablation) at various postoperative intervals. Enzyme activity expressed as* 10 *µl O per hour*
Mean value ± S.E.M. N.S. = not significant.

Time after operation	N of animals	Left side: No. of cells	Left side: Mean value ± S.E.M.	Right side (operated): No. of cells	Right side (operated): Mean value ± S.E.M.	P
1 hour	4	11	4.8 ± 1.1	7	6.8 ± 0.5	N.S.
7 days	5	10	9.5 ± 1.5	12	5.3 ± 0.7	0.01
15 days	4	9	6.1 ± 0.4	11	3.2 ± 0.3	0.01
40 days	5	12	4.1 ± 0.5	9	4.1 ± 0.6	N.S.

system results in a complex pattern of compensatory mechanisms, and the following offers a tentative explanation of the background to the observed asymmetrical enzyme changes Impulses from the cerebellar cortex impinging upon the Deiters cells depresses the activity in these cells (Ito & Yoshida, 1964 1966) and removal of this inhibition can thus be regarded as a stimulation resulting in increased cellular respiration. This explains the increased succinoxidase activity in Deiters giant cells on the right side where hemicerebellectomy was performed A small increase was registered also on the left side and this may be due to a minor injury to the remaining half of the cerebellum and/or an unspecific action of the operation trauma as such.

Shimazu & Precht (1966) demonstrated the impulses between the vestibular nuclei of both existence of a pathway mediating inhibitory sides. It is thus possible that the shift in functional overweight from the right to the left side, as judged by the cytochemical observations, is due to inhibitory impulses originating from neurons in the left vestibular nuclei. When total cerebellectomy was performed (Blomstrand et al., 1968 *a*) the succinoxidase activity identical in the Deiters neurons from both sides, with high values at 7 and 15 days and almost preoperative values at 40 days postoperative This is in good accord with the observations made on the left side in the present study and indicates the unilateral nature of the operation

Determination of RNA content in the Deiters cells after hemicerebellectomy did not show the same degree of asymmetry between the two sides The slightly lower RNA content in isolated cells of the operated side compared to the contralateral side at 7 and 15 days parallells the bigger side difference in the same direction for succinoxidase activity in this cell type However since the curves of RNA values against time do not have significant side differences, they are more similar to what was observed after total cerebellectomy

In several earlier studies the reactions of RNA and respiratory enzymes have been fairly parallel after changed neuronal function (Hydén & Pigon, 1960 Blomstrand et al., 1966 *a*), but in some experiments these parameters have shown opposite changes (Blomstrand et al., 1968 *a*, *b*) In the present work there are obvious qualitative differences between the two suggesting different functional reactions to a new neuronal activity The succinoxidase activity of a nerve cell has been shown to reflect the amount of mitochondria (Eneström & Hamberger 1968) while the RNA content reflects the protein synthetizing capacity Mitochondria are in part autonomous organelles of a living cell why it is not surprising that these two biochemical parameters do not react in exactly the same way after a certain functional influence. However the curves of cytochemical changes against time seem to be analogous in one respect. The succinoxidase activity and RNA content are both almost restored to the preoperative value after 40 days and this time course is parallel to those after unilateral neurotomy (Blomstrand et al., 1966 *a*) and total cerebellectomy (Blomstrand et al., 1968 *a*). Imbalances, induced in different ways, between the vestibular nuclei of the two sides cause changed cytochemical activities in the giant nerve cells of the lateral vestibular nucleus, the nature of the change depending upon the type of imbalance Regardless of the disturbance imposed upon the system, there seems to be approximately the same time course for restitution of the cytochemical changes back to normal This might reflect compensatory mechanisms in the cellular units in order to adjust the vestibular system to a new balance between the two halves of the system.

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ZUSAMMENFASSUNG

In einzelnen Deitersschen Riesennervenzellen vom lateralen Vestibularkern bei Kaninchen, welche Hemicerebellectomie unterworfen waren, wurden die Menge an RNA und die Succinoxidaseaktivität gemessen. Nach der Operation erhielt man eine abnehmende RNA Menge bilateral nach 1 2 Wochen und beinahe wieder hergestellte Werte nach 6 Wochen. Die Succinoxidaseaktivitat zeigt asymetrische Veränderungen mit anfänglich höheren Werten auf der operierten Seite und mit einer Steigerung auf der contralateralen Seite nach einer Woche. Die Succinoxidaseaktivität und RNA Menge kommt später zurück zu den ungefähr voroperativen Werten.

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THE VESTIBULAR EFFECTS OF MECLIZINE HYDROCHLORIDE-NIACIN COMBINATION (ANTIVERT)

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Abstract Objective, electronystagmography monitored positional, caloric, rotational or linear acceleration vestibular tests were carried out on 30 normal subjects and 30 subjects with vestibular disease before and after administration with meclizine hydrochloride-niacin combination or placebo. The authors found the otolithic and semicircular canal functions to be most effected. Side effects were few and minor This drug seems very useful for treatment of ambulatory patients with vestibular disease. The effect on vestibular function of normal individuals was noted only during linear acceleration.

The principles of the torsion swing have been [illegible]ed by Mach (1875) and recently by various European authors. (Van Egmond et al. 1943 Groen & Jongkees, 1948 Hennebert, 1961 Greiner et al. 1963 Boer et al. 1963 Pignataro & Dittrich, 1963 Sokolovski 1963) When used in combination with electronystagmography (ENG) one can determine whether there is at least one excitable labyrinth and whether a directional preponderance of the vestibular nystagmus exists (Oosterveld, 1965)

The sensitivity of the vestibular system to linear acceleration can be measured with the aid of the parallel swing. The otolith mechanisms of the utricle and saccule are the suborgans of the peripheral vestibular system which are sensitive to linear acceleration (Jongkees & Philipszoon, 1964).

Sixty subjects were examined using electronystagmographically monitored vestibular tests. Group A consisted of 120 normals and 10 patients with vestibular disease. Baseline values were determined for positional nystagmus, bithermal caloric responses and reaction to angular acceleration on the torsion swing. Each subject was given either "Antivert or placebo t.i.d. for 1 day Identical vestibular testing was carried out 1 hour after the third dose Ten normals were given placebo, 10 normals were given Antivert" and the 10 patients were all given Antivert" Neither the patients nor the investigator knew who had received the placebo

Group B consisted of 10 normals and 20 patients with vestibular disease. After studies to determine the presence or absence of positional nystagmus, baseline values for responses

Subject material

Diagnosis	No. of subjects
Normal test persons	30
Menière's disease	16
Vestibular neuronitis	3
Chronic otitis media	3
Post head trauma	3
Acoustic neuroma	2
Post meningitis	1
Labyrinthine ischemia	1
Benign paroxysmal vertigo	1
	60

This study was supported by a grant from the J. B. Roerig Division, Charles Pfizer and Company

Table I *Bithermal caloric test*

Percentage change from baseline values 1 hour after administration of meclizine HCl–niacin combination.

Group A	Speed (°/sec)	Duration (sec)
10 normal subjects given Antivert	−2.2%	−6.0
10 patients with vestibular disease given Antivert	−14 1	−4.5
10 normal subjects given placebo	−0.5	−7 6

Statistically significant change.

Table II *Torsion swing test*

Percentage change from baseline values 1 hour 45 minutes after administration of meclizine HCl–niacin combination

	Frequency (beats)		Duration (sec)	
Group A	Left	Right	Left	Right
10 normal subjects given Antivert	−5 1	−0 4	−11.3	−13 4
10 patients with vestibular disease given Antivert	−0.3	−3.1	−11 7	−9.3
10 normal subjects given placebo	−3.3	−1 4	+1 4	−5.0

Statistically significant change.

to linear accelerations on the parallel swing were determined. Using a double-blind method, half of the normals and half of the patients in Group B were given Antivert The other 15 subjects received placebo

RESULTS

Table I lists the average percentage change recorded in 30 individuals given either Antivert" or placebo The bithermal caloric test proved not to be a sensitive indicator of drug effect on the vestibular system Only the normal placebo group had a statistically significant change in nystagmus *duration* (−7 6%) There was essentially no change in the speed of the slow component of nystagmus (the most reliable parameter for caloric nystagmus)

Angular acceleration (torsion swing) is a more physiologic means of stimulating the semicircular canals. In our study there was no statistically significant change in the *frequency* of nystagmus for any of the 30 subjects tested on the torsion swing. There was however a significant decrease in the *duration* of nystagmus (the most constant parameter), (Oosterveld, 1967) in both directions, for the group of 10 patients with vestibular disease who were given Antivert' A similar decrease in duration of nystagmus to the *right* only was noted in the normal subjects who were given Antivert Other authors (Boer et al., 1963 Crampton, 1962 Fluur & Mendel, 1963) have reported similar individual-linked directional preponderance" in normal individuals. Only *the subjects with vestibular disease had a significant decrease in both directions* (Table II)

Linear accelerations provoked by the parallel swing stimulate the otoliths. Ten subjects with vestibular disease given Antivert" had a significant decrease in amplitude of parallel swing provoked eye movements. Five normal test persons given Antivert" had an even greater though less sustained, decrease in provoked eye movements (Table III) Ten other patients with vestibular disease who were given placebo had no significant decrease in amplitude of eye movements. The 5 test sub-

Table III *Parallel swing test*

Percentage change in amplitude of eye movements elicited by parallel swing test 30, 60, 90, minutes after administration of meclizine HCl–niacin combination. All figures represent mean ±S.E. There is statistically significant decrease in eye movement noted in all subjects who received meclizine HCl–niacin combination.

Group B	60 minutes	90 minutes	120 minutes
5 normal subjects given Antivert	−17.8 ±2.1	−20.4 ±2.3	−20.8 ±1.5%
10 patients with vestibular disease given Antivert	−11 4 ±1 4%	−11 9 ±2.8%	−15.9 ±3.5%
5 normal subjects given placebo	0.6 ±2.2%	0 2 ±1.9	0%
10 patients with vestibular disease given placebo	−0.1 ±1.0%	−2.7 ±1.5%	−2.5 ±1 7%

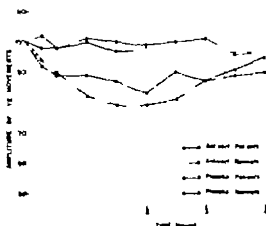


Fig 1

jects given placebo likewise had no significant change in eye movements The most profound depression of eye movement amplitude oc curred between 1 1/2 and 2 hours following ingestion of "Antivert" (Fig. 1)

Positional nystagmus was not affected in any way by either Antivert" or placebo Those subjects with positional nystagmus before med ication had the same positional nystagmus when tested later Test subjects without posi tional nystagmus did not exhibit any as a result of the drug.

Side effects occurred in 22% of subjects en Antivert" and 15% of subjects given placebo. Ten persons complained of drowsiness, one subject had dryness of the mouth, one had blurred vision and one became flushed and drowsy

DISCUSSION

Through the use of ENG monitored multiphasic vestibular testing, the effects of meclizine hydrochloride–niacin combination (Anti vert) on vestibular function can be objectively determined.

In the above study polyparametric criteria for detection of change in the function of the utricles and semicircular canals, before and after the ingestion of medication or placebo were applied. It was again determined that the bithermal caloric test is too gross and over stimulating to be of value in sensitive drug studies Torsion swing induced angular acceleration and parallel swing induced linear acce leration tests are exceptionally useful for test ing the effects of drugs on the vestibular system in ambulatory subjects.

In view of the low incidence of side effects at therapeutic dosage and the fact that the most significant reductions in vestibular excitability occurred on stimulation by linear and angular acceleration and that these stimuli are the most frequently encountered during locomotion, meclizine HCl–niacin combination should be very useful in the treatment of ambulatory patients with vestibular disease.

ZUSAMMENFASSUNG

Objektive und mit ENG registrierte Vestibularprüfungen (Lage kalorisch, Drehung, oder lineare Beschleunigung) wurden an 30 normalen Personen und an 30 Patienten mit vestibularen Störungen durchgeführt und zwar vor sowie nach der Gabe von Meclizine Hydrochloride–Niacin Kombination oder Placebo. Die Autoren fanden die otolithischen und die Bogengangsfunktionen am meisten verändert. Nebenerscheinungen waren selten und geringfügig. Diese Droge scheint sich gut zur Behandlung von ambulanten Patienten mit vestibularen Leiden zu eignen. Der Effekt auf die vestibulare Funktion bei normalen Personen war nur während der linearen Beschleunigu g bemerkbar

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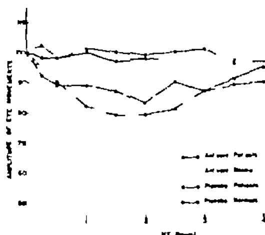


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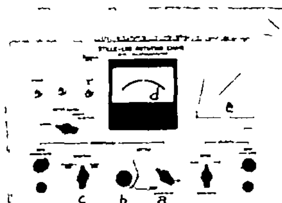


Fig. 1 The central unit of the Stille-LKB rotating chair. By setting knobs (*a*) and (*b*) the acceleration can be preset. By turning knob (*c*) this acceleration is terminated when the ammeter (*d*) has reached the desired value plotted from the nomogram ( ).

nies (1932) met more advanced requirements and was mainly used to study postrotational nystagmus according to the method that had come to be known as the Buys–Fischer principle. Interference caused by rotational nystagmus is eliminated in this method by making use of an extended period of subliminal acceleration up to the level of constant speed, at which level the braking procedure is then commended. Among others, Tönnies (1932) Woletz (1933), Arslan (1934) and Mittermaier (1950) have worked along these lines.

Different types of rotational test have been developed in different quarters. Cupulometry with subliminal acceleration attaining different angular velocities followed by sudden deceleration, was developed by the Dutch school with van Egmond, Groen & Jongkees as representatives (1948, 1949). In Sweden, as in other countries, a symmetrical acceleration–deceleration test has come into use during the last few years (Inter alia, Stahle, 1958). In their investigations employing the "catapult start" and the "catapult stop" with very pronounced accelerations and decelerations effected almost instantaneously Mittermaier and his team equipped their rotating chair with a magnetic coupling between the drive shaft of the motor and the rotation shaft of the chair. Essentially modern rotating chairs are based on the design presented by Hallpike, Hood & Byford in a comprehensive study in 1952. This design meets highly advanced technical requirements with respect to precision and manoeuvrability. It provides a large number of values for acceleration and deceleration as well as a wide range of variations with respect to constant velocities and times for application of the stimulus.

A Swedish chair based on the same principles was introduced by Stille–Werner on the initiative of the late Prof. P. Frenkner. The project commenced with consultations between S. Malmström of LKB Produkter AB on behalf of Stille–Werner and C. J. Hallpike in London. L. Wegstedt and S. Henell, engineers at LKB Produkter AB worked out the Swedish design. The older system consists of: *Rotating chair* with base driving unit and seat with wall suppors, a *Power unit* and servo system and a *Central unit* (Fig. 1) with servo amplifier. (See Frenkner & Preber 1956.)

The later model of the base and driving unit is shown in Fig. 4 (a modification initiated by Fluur). The installation consists of a base with a driving unit (motor-generator), a seat with attachment for the patient and wall supports. In addition to the rectifier unit itself, the power unit also houses the second motor-generator of the servo system as well as the servo potentiometer. The central unit houses two servo amplifiers for the two motors in addition to all necessary control equipment.

Disadvantages of the installation used to date

Up to now the chair has been equipped with semi-automatic control facilities. After preselecting values for positive and negative acceleration (buttons *a* and *b* Fig. 1) changeovers to constant rotation and stopping have been effected manually by controlling the angular velocity of the chair by means of button *c* and an ammeter (*d*) (Fig. 1). Considerable practice and attentiveness have been required to obtain satisfactory values, particularly the correct value when changing over from con-

stant acceleration to constant angular velocity When decelerating it has probably been easier to watch the chair instead of the ammeter to determine the cessation of rotation and then on the basis of this observation, reset the control button for negative or positive acceleration (c Fig. 1) The time intervals for constant speed and standoff have often been irregular

Objectives set for the new design of the program unit

The desired objective which was defined by M. Bergstedt, has been to design a program unit that will make it possible to plan one or more test cycles with alternating clockwise and anticlockwise rotation following the patterns of a number of common rotation test methods. Another objective has been to render the duration of the stimulus (acceleration and deceleration) as exact as possible Since a cupulogram should embrace three levels" of constant speed from which deceleration is started and includes both clockwise and anticlockwise rotation, six cycles may be preset. If the same cycle is to be repeated a number of times, h repetition is effected by means of the ·ater mechanism.

Acceleration and deceleration values

These values for the program unit are fixed, ranging from 0 4 /sec² to 14 /sec² Other values are obtainable on request. The values for acceleration and deceleration have been chosen with respect to the threshold values" of the cupula organ, which usually indicates 0.3–0.5 /sec for sensation and 0 8–1 0 /sec for nystagmus observed by ENG If other values are desired they are effected "by hand by disconnecting the program unit and setting the acceleration control knob for acceleration and selecting the desired value.

The times for constant velocity and standoff have been selected in consideration of the most usual conditions for rotation tests, e g. from 30 sec to 13 min.

If on deceleration only nystagmu is to be studied there is a relay that switches on the printer 10 sec b fore deceleration, and the recording function remains in effect as long as the chair is at a standstill

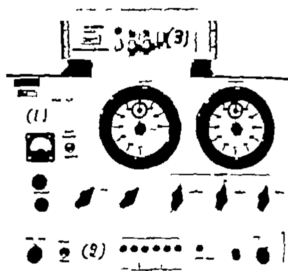


Fig 2 The new program unit designed for the Stille-LKB rotating chair (1) Program unit RS-22, (2) Control unit RS-22 and (3) Preset counter The central unit in Fig. 12, the power and servo unit and this new program unit constitute the program device for the latest model of the Stille LKB rotating chair.

Design and construction of the new program unit

The latest model of the Stille LKB rotating chair consists of three basic units (Fig. 2): a *program unit* (1) a *control unit* (2) and a *preset counter* (3) In addition there is a common power unit for all three basic units (Same construction as in earlier design see unit (2) in figure)

The program unit—which is the central unit—is fitted with a timer for setting standoff time as well as the period for constant angular velocity The preset periods range from 30 sec to 13 min The program unit is also equipped with control knobs for the step-by step setting of acceleration and deceleration values from 0 4 up to 14/ sec² With the aid of three separate controls, the constant angular velocity for six different cycles can be programmed in advance The control knobs can be used to select constant velocities from 15 /

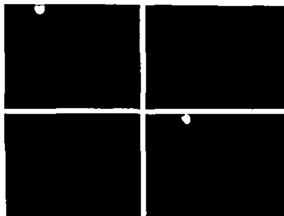


Fig 3 Oscilloscope pictures of the servo voltage from the power unit to the driving motor f the Stille-LKB rotating chair Osc. sweep = 2 sec cm and Y = 5 V/cm. The two upper pictures show accelera tion from 0 to 60°/sec and the lower two decelera-tion from 60 to 0°/sec, all at 6 /sec² The two pic tures on the left are *without* and the two pictures on the right are *with* the load f a test subject of 150 pounds. Note the straight lines and the similarity f the four tests.

sec up to 150 /sec. Lastly the program unit is fitted with a selector switch for clockwise or anticlockwise rotation. Impulses are sent out from the program unit to a present counter mak ing it possible to complete up to 100 cycles in succession.

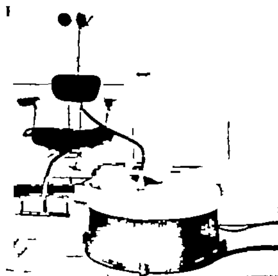


Fig 4 Later model of base and driving unit of the Stille-LKB rotating chair Modification by E. Fluur (1964).

There is a two-way connection lead from the program unit, (1) in Fig. 2, to the *control unit* (2) Mounted on the latter are a selector switch for manual or automatic control, controls for starting and resetting, and controls for single or repeated test cycles. The control unit is fitted with a set of pushbuttons making it possible to program a number of test cycles in advance Each cycle consists of accelera tion in the desired direction and the desired deceleration down to zero velocity If the installation is programmed for an additional cycle, the chair remains at rest during the preset standstill period, whereupon a new cycle is begun in the opposite direction. In order to effect very rapid decelerations, the rotating chair has been fitted with an elec trically controlled hydraulic brake which is actuated by a signal from the control unit. Control signals are also sent out from the con trol unit to the recording mechanism. This control has been provided to save printer paper If so desired the printer is then in operation only during the interesting portion of the cycle and remains at rest during the meantime It is possible for the patient to interrupt a cycle and bring the velocity down to zero in case of nausea or any other complaint. This is ef fected by means of a connecting lead from the patient via the sliding contacts of the chair to the control unit. The magnitude of the deceleration the chair is to undergo if the patient experiences nausea can be preset. The control panel is also fitted with a reset but ton which operates in parellel with the pa tient's stop button. The control unit is connected to the regular program equipment already used earlier If so desired, the chair can be operated by means of the old control unit only (Fig. 1)

A schematic picture of the rotating chair and the control units is presented in Fig. 4

Control of the rotating chair by means of the new programming unit

Checks made to date indicate that the cor relation between actual values and preset val

ues is very good. One of the methods employed to check the values was to connect a speedometer to the rotating chair; the other was to measure the servo voltage supplied to the chair motor. Correlation between these values is found to be very good (Fig. 3).

In order to study the advantages of automatic operation of the chair vis-à-vis manual operation, a series of measurements was performed with different values for acceleration and deceleration respectively. The results calculated for automatic control show very good consistency between the different runs with a deviation of less than 0.5%. For the check on manual operation, we let laboratory technicians do a series of runs. *After training they were instructed to try to attain the highest possible consistency for similar runs.* The difficulty in obtaining consistent values during manual operation is clearly evident: the results reveal a percentage error of 2.5%, i.e., 5 times the value for automatic control.

In addition to the advantage of reducing the percentage error gained by automatic control vis-à-vis manual operation, there is also considerable simplification of the whole opera procedure. As mentioned above, great and intimate knowledge of the apparatus was required before now and the operator was completely occupied by the apparatus throughout the entire procedure. With automatic control it is only necessary to start the program mechanism and the entire operation is performed automatically and precisely without human intervention at any point in the procedure. This makes it possible for the operator to devote his time to the printer and—on the basis of the printed record—monitor the test, noting acceleration times, velocities, etc., all of which amounts to a tremendous simplification. To our mind, it is clearly evident that the described programming unit constitutes an essential improvement in the usefulness of the chair in research and simplifies the routine examination of patients considerably.

ZUSAMMENFASSUNG

Der Artikel beschreibt unter anderem die Entwicklung des Drehstuhles für vestibuläre Untersuchungen entsprechend den Entwicklungsrichtungen Bárány–Fischer–Tönnies–Hallpike einerseits und Frenkner–Preber–Fluur andererseits. Eine Weiterentwicklung des bekannten Stille LKB-Drehstuhles wird vorgestellt. Sie enthält eine automatische Programmierung, welche aus den folgenden 3 Grundeinheiten zusammengefügt ist: Programmeinheit, vorgeschaltete Zähleinheit und an die Antriebseinheit gekoppelte Kontrolleinheit. Diese Programmeinheit ermöglicht die Vorwahl einer bestimmten Anzahl von Testläufen (sechs), wobei praktisch eine unbegrenzte Vorwahl *und Verknüpfung von Beschleunigungen,* Entschleunigungen und Zeitabständen sowie konstanter Geschwindigkeiten und ihrer Zeitwerte einstellbar ist. Zusätzlich ist der Stuhl mit einer elektrisch gesteuerten hydraulischen Bremse versehen, die schnelle Entschleunigungen gestattet. Ein vorgeschaltetes Zählwerk erlaubt den Ablauf von Tests mit vielen ähnlichen Steuerschritten.

Im Gegensatz zur manuellen Bedienung bietet die automatische Steuerung den großen Vorteil, daß einerseits der Prozentsatz an Bedienungsfehlern erheblich reduziert wird und daß andererseits eine außerordentliche Vereinfachung bei der gesamten Durchführung solcher Tests bewirkt wird.

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ETUDE DES RYTHMES DU BATTEMENT CILIAIRE

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(Reçu 4 Septembre, 1969)

Resumé Après avoir souligné l'importance de l'étude objective du mouvement ciliaire qui constitue à la fois un test pharmacodynamique et un index précis de l'état physiologique des cellules ciliées, les auteurs rapportent leurs expériences concernant trois méthodes d'étude) la Stroboscopie, *b)* l'enregistrement cinématographique en prise de vues accélérées, *c)* la microphotooscillographie. Cette dernière méthode est une amélioration de la méthode photooscillographique proposée par Dalhamn et Rylander en 1962. Les battements d'un seul cil sont capables de moduler le flux de lumière et permettent un enregistrement, soit graphique, soit photographique. Un micro-enregistreur de température est couplé au système Les résultats obtenus par ces différentes méthodes sur de nombreux épithéliums ciliés sont analysés et comparés.

L étude de l'activité ciliaire offre de multiples intérêts. C'est, tout d'abord, un intérêt d'ordre pharmacodynamique sur lequel nous n'insisterons pas, sinon pour signaler qu actuellement, en France, tout solution ou suspension destinée à la thérapeutique locale au niveau des fosses nasales et des voies aériennes supérieures doit être soumise à des tests précis concernant son action sur l activité des cils vibratiles. Seuls les produits thérapeutiques qui ne modifient pas le battement ciliaire, après 20 minutes de contact avec l éphithélium, sont admis dans l arsenal thérapeutique. C'est souligner l importance physiologique du battement ciliaire qui assure, par le drainage du mucus qu'il entraîne, la stérilité et l'auto-épuration des voies aériennes supérieures.

Sur le plan de la recherche cytologique l'activité ciliaire constitute un index d une parfaite fidélité de l'état biologique des cellules épithéliales qui constituent le substrat anatomique de ce mouvement. Ainsi peut-on, de façon relativement simple, étudier soit en culture cellulaire soit, plus simplement, après mise en survie les réactions cellulaires à de multiples modifications du milieu ambiant. L anaphylaxie locale trouve ici un champ d étude particulièrement intéressant puisqu'il s agit, pour la grande majorité des substances anaphylactiques qui sont inhalées par les mammifères (pneumallergènes) du premier type cellulaire avec lequel elles entrent en contact.

Enfin si l unité des structures ciliées, des protistes aux vertébrés, a été clairement mise en évidence, depuis vingt ans, grâce aux études morphologiques rendues possibles par le microscope électronique, nos connaissances en ce qui touche aux sources d'énergie utilisées par le battement ciliaire et, de façon plus générale les conditions biochimiques de ce battement sont loin d'être élucidées

En tout état de cause, la première des con-

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ditions nécessaires, si l'on veut utiliser pleinement l'excellent index biologique que constitue le battement ciliaire dans des études aussi bien de recherches fondamentales qu'appliquées, est de disposer d'une ou plusieurs techniques précises qui permettent d'étudier les modifications éventuelles de ce rythme. En l'absence de telles techniques, on est, en effet, condamné à ne prendre en considération que les variations expérimentales les plus grossièrement évidentes de ce rythme, et même, à l'extrême, à n'envisager que la loi du tout ou rien, c'est à dire la persistance ou l'arrêt total du battement dont les constatations sont évidentes, mais qui privent l'expérimentateur des observations les plus fines sur tous les stades intermédiaires possibles entre le rythme normal et l'arrêt total. L'idéal serait une technique qui, en des conditions expérimentales parfaitement reproductibles du point de vue physique et biochimique (température, éclairement, isotonicité du milieu, condition de pH), permettrait d'étudier les paramètres du mouvement ciliaire, qui sont la fréquence du battement, son amplitude, sa direction, sa force ou le travail qu'il peut effectuer la longueur d'onde du rythme métachrone, la vélocité des ondes transmises au mucus.

Or une telle technique n'existe pas, et même si l'on se contente d'étudier comme nous l'avons fait, les deux premiers paramètres, c'est à dire fréquence et amplitude du battement, force est de reconnaître que les solutions proposées ne sont pas pleinement satisfaisantes. L'analyse des méthodes proposées par les nombreux chercheurs qui ont étudié ces problèmes nous permettra de préciser cette critique

On peut distinguer deux grands types de méthodes d'étude de l'activité ciliaire, 1) les méthodes directes qui nous intéressent précisément ici, et 2) les méthodes indirectes, qui furent les plus anciennement utilisées pour étudier globalement l'activité ciliaire

Ces dernières peuvent se résumer ainsi.

(*a*) la simple observation, sous loupe binoculaire, de la progression de particules très légères (talc, carbone, poussière de métaux légers) entraînées par le mucus sous l'effet du battement ciliaire

(*b*) L'emploi de différents petits appareils pour mesurer directement le travail réalisé par le mouvement ciliaire c'est le principe du cylindre de verre de Chamil (1881) du Flimmermühle de Buchner (1877) ou du ciliоscribe de Inchley (1921)

Ces méthodes peuvent, certes, avoir un certain intérêt, lors d'études pharmacodynamiques en particulier mais elles sont entièrement inapplicables, en raison de leur caractère rudimentaire et global, à l'étude du mouvement ciliaire en tant qu' index biologique

Les méthodes directes visent toutes à une mise en évidence directe des modifications du rythme et de l'amplitude des battements ciliaires et, à ce titre, elles nous intéressent beaucoup plus que les précédentes.

La technique la plus simple est évidemment d'observer sous microscope le battement ciliaire Mais dans sa forme la plus simple (observation entre lame et lamelle) elle introduit un certain nombre de causes d'erreurs ou d'inconnues non mesurées. C'est, essentiellement, la température exacte de la préparation lors de l'observation microscopique c'est aussi le fait de l'anoxie tissulaire incontrôlable c'est enfin, et surtout pour les animaux marins, une évaporation qui, compte tenu de la faible quantité de liquide utilisée pour l'observation microscopique, provoque rapidement une augmentation de la salure du milieu, très toxique pour la survie cellulaire Cette méthode condamne donc l'observation à une extrême brièveté — la plupart des auteurs limitent la durée de chaque observation à 30 secondes.

Nous avons apporté à cette méthode une modification technique qui pallie les différents défauts indiqués précédemment. Nous remplaçons la lamelle par une mince couche de plasma que l'on fait coaguler par la thrombase et qui enferme dans le caillot le fragment d'épithélium cilié, lui-même en suspension dans une goutte de liquide approprié (eau de

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utilisant un appareil de stroboscopie électronique.

Nous avons utilisé, pour la présente étude, le stroboscope type PR 9104 (Philips) Cet appareil est destiné par son constructeur à une utilisation industrielle, mais son adaptation aux nécessités de l observation microscopique est possible. La lampe dont il est doté est une lampe à vapeur de mercure lampe à relaxation qui fournit une intensité lumineuse moyenne (de 200 lux) dans les fréquences qui nous intéressent. La précision de l'appareillage, suivant les normes du constructeur est de ±2% entre 5 et 200 cycles/seconde. Dernier paramètre physique, enfin, mais il est d'importance capitale dans le domaine de la stroboscopie, la durée des éclairs est de 10 micro/seconde Il ne peut donc y avoir ici de traînées lumineuses lors de l'observation visuelle

Méthodes et matériel

Nous avons utilisé soit un microscope Zeiss, type Optovar équipé pour examen en contraste de phase, les grossissements obtenus étaient alors au maximum de 800 diamètres, soit un microscope interférentiel Nachet, les grossissements obtenus étant alors de l'ordre de 500 diamètres, en raison de l'emploi d'oculaires moins puissants. L emploi du contraste de phase (ou de la microscopie interférentielle) est indispensable lors de telles recherches, sinon le caractère aveuglant de la lumière et l'absence de relief de l image obtenue ne permettraient ni de distinguer les cils individuellement, ni même d'apprécier nettement leurs battements.

Température

Il est certes difficile, nous l avons vu, de fixer avec précision la température Lors de l'examen microscopique proprement dit, nous avons mesuré, grâce à un thermocouple, en fin d'expérience la température du milieu de survie dans lequel étaient immergés les fragments d'épithélium soumis à l examen. Nous avons trouvé des valeurs qui variaient de 18 à 26° et il est à noter que la lumière émise par la lampe de l'appareil stroboscopique est une lumière froide ou très peu calorique Nous verrons plus loin que ce problème peut être résolu par l'emploi d'un microthermocouple

Technique d'observation

A ce propos, nous avons utilisé deux techniques différentes d'examen microscopique

La première consistait à individualiser un seul cil, puis à amener la fréquence des éclairs du stroboscope jusqu à une valeur telle qu on puisse, durant 30 secondes, compter les battements individuels ainsi observés au ralenti. A titre de contrôle la fréquence des éclairs était ensuite portée à une valeur telle qu elle réalisait un éclairage optiquement continu de la préparation, ceci afin de s assurer que le battement examiné était réellement persistant et d'une fréquence constante, dans les limites étroites du temps d'observation et de comptage Dès lors, on peut affirmer en ce cas particulier que la fréquence du battement est celle de la fréquence stroboscopique augmentée, si l on suit l'échelle ascendante des fréquences, du nombre de battements comptés par l'observateur

Cette première méthode ne peut s adresser qu'à des structures ciliaires particulièrement longues, d'une part, et d'implantation assez espacée, d'autre part, pour autoriser la sélection visuelle d'un cil isolé. C'est le cas pour un petit nombre de cellules ciliées, en particulier celui des cils latéraux frontaux de *Mytilus edulis* matériel particulièrement favorable du fait de sa disposition anatomique et de la stabilité optique de la préparation Ensemble de faits qui expliquent que la grande majorité des auteurs se soient contentés de ce matériel lors des études stroboscopiques sur le mouvement ciliaire.

La nécessité où nous nous trouvions d'effectuer une enquête beaucoup plus vaste, portant sur le maximum d'animaux ciliés, nous a obligés à recourir à une deuxième technique. On se trouve très fréquemment, en effet, devant des structures ciliées implantées en touffes et dont les battements apparaissent singu-

lièrement intriqués. Circonstances qui s'aggravent encore du fait que nous ne nous sommes pas contentés d'examiner des battements ciliaires extérieurs à l'animal, mais également ceux dont sont le siège certaines cellules des organes internes, examinés dès lors par transparence (tels, par exemple, les néphridies d'oligochètes). En ce cas, il est à peu près impossible d'individualiser un cil: nous avons donc choisi des zones aussi étroitement limitées que possible et dont le battement apparaît synchrone. On amène la fréquence des éclairs du stroboscope à une valeur telle que l'ensemble apparaît à peu près immobile mais il serait vain, d'après ce que nous avons indiqué précédemment en de tels cas, de rechercher une stricte immobilité de l'ensemble. Une telle mesure, inhérente aux facteurs anatomiques eux même peut entraîner une certaine erreur dans l'appréciation du rythme puisqu'en somme on ne mesure plus la vitesse d'un cil, mais d'une touffe de cils dont on est jamais très certain que ses éléments ne se déphasent pas en cours de mesure.

D'autre part, les difficultés majeures dans la mesure du rythme des battements sont de plusieurs ordres

1) la première est d'ordre physiologique. Elle est constituée par la très inégale résistance des structures ciliées, suivant les divers embranchements étudiés et bien que toutes précautions soient prises pour utiliser des liquides de survie les mieux adaptés. C'est ainsi qu'alors que les cils de la muqueuse des voies aériennes supérieures des mammifères continuent de battre durant de nombreuses heures après excision, les cils de certains animaux marins, transportés en dehors de leur milieu habituel, peuvent cesser de battre en moins de 20 minutes. C'est dire d'une part, la nécessité d'opérer chaque comptage du rythme ciliaire le plus rapidement possible (à ce propos, la souplesse d'utilisation du stroboscope électrique est remarquable) et, d'autre part, la nécessité d'adapter des techniques spéciales pour certains animaux, en particulier invertébrés marins auxquels il convient d'assurer un courant d'eau constant à une température correctement adaptée.

La seconde difficulté est d'ordre physique, il s'agit du phénomène des harmoniques stroboscopiques. Un exemple chiffré fera mieux comprendre le phénomène. Admettons qu'un cil offre un rythme de base de 400 coups/minute, on obtiendra l'arrêt stroboscopique non seulement avec cette fréquence, mais également avec ses harmoniques, c'est à dire 800 etc. En fait, c'est surtout le premier harmonique qui introduit une cause d'erreur importante. Il convient donc de pratiquer ces examens suivant une technique précise.

a) On effectuera un balayage des fréquences, en commençant par une fréquence élevée (1600 cycles/minute, par exemple) et en la diminuant progressivement.

b) En se basant sur ces premiers résultats, on effectuera un nouveau balayage des fréquences, en commençant par la valeur la plus basse possible, compatible avec une observation correcte. Ce balayage est difficile, les fréquences inférieures à 400 coups/minute offrent évidemment des temps obscurs considérables et l'œil risque alors de perdre la structure étudiée. C'est dire que c'est dans cette zone comprise entre 300 et 400 coups/minute que les mesures sont les plus délicates, car c'est, en effet, la zone limite des possibilités de la stroboscopie ou les mesures, du fait de la persistance de l'image rétinienne, provoquent un éblouissement vite insupportable de l'œil (fréquence épileptogène).

Nous avons, lors de ce premier bilan, tenté d'étudier la plus grande variété possible de ciliés, mais il est bien évident qu'on ne saurait en ce domaine être complet.

Ces résultats furent obtenus à la Station de Biologie marine d'Arcachon par deux observateurs qui travaillaient indépendamment l'un de l'autre et confrontaient leurs résultats, lesquels concordaient à peu près constamment.

Cette longue expérimentation nous rend d'autant plus facile la critique de la méthode stroboscopique. Ces résultats, en effet, sont à peu près tous faux, ainsi que la cinémato-

ÉTUDE STROBOSCOPIQUE

Protozoaires			
ciliés psammophiles	*Euplotes eurystoma*		700 cycles/minute
	Opalina ranarum	membranelles	650–700 cycles/minute
Cnidaires			
hexacoralliaires	*Anemonia sulcata*	paroi septale	360–400 cycles/minute
Plathelminthes Turbellariés			
acoeles	*Convoluta schultzei*	ciliature externe	480–500 cycles/minute
Aschelminthes			
rotifères gastrotriches	*Brachionus pala*	ciliature externe	360–400 cycles/minute
	Tetranchyroderma massiliense		
Bryozoaires		cils tentaculaires et stomachaux	700–800 cycles minute
Sipunculiens			
	Sipunculus nudus	intestin	470–450 cycles/minute
Annélides :			
polychètes	*Eulalia viridis*	parapodes	360–400 cycles minute
	Nephtys hombergii	branchies	360–400 cycles/minute
oligochètes	*Marionina achaeta*	parapodes	800 cycles minute
archéannélides	*Protodrilus symbioticus*	parapodes	500 cycles minute
Mollusques			
polyplacophores	*Acanthochiton*	intestin	400 cycles minute
bivalves	*Mytilus edulis*	branchie	350–400 cycles minute
gastéropodes opisthobranches	*Aplysia depilans*	vésicule biliaire	400–450 cycles minute
Echinodermes			
	Asterias rubens	branchies	450–500 cycles minute
Tuniciers urocordés			
	Ciona intestinalis	branchies	400–420 cycles minute
	Ascidia aspersa	branchies	400–420 cycles minute
	Botryllus schlosseri	intestin	700 cycles minut
Céphalocordés			
	Branchiostoma lanceolatum	épithélium branchial	650–700 cycles minute
Vertébrés anoures			
	Rana temporaria	pharynx	400–450 cycles/minute
Vertébrés homéothermes			
	Lapin	trachée	700–750 cycles/minut

graphie en prise de vues accélérées nous le montra ultérieurement. Si la stroboscopie représente la méthode de choix pour mesurer avec exactitude le nombre de cycles par unité de temps décrits par un système mécanique qui repasse toujours exactement par son point d'équilibre (et décrit donc un trajet fixe), il n'en est pas de même pour un système biologique, tel que le mouvement ciliaire, qui ne peut être comparé au mouvement d'un métronome. D'une part, on sait que le mouvement ciliaire se décompose en deux phases de durée inégale (qui conditionnent, d'ailleurs, la possibilité pour un tel mouvement de déplacer liquides et particules) une phase rapide, active, et une phase de rappel, plus lente, inactive ou passive, durant beaucoup plus longtemps que la phase active. D'autre part, il apparaît que le cil ne bat pas strictement dans un plan idéal sans épaisseur mais, surtout en ce qui concerne les cils les plus longs, sa pointe dessine, en vue supérieure, une sorte de 8 allongé.

Enfin, l'étude stroboscopique fait appel à la collaboration de l'œil de l'observateur et nous nous sommes vite persuadés que l'œil était incapable de constituer un système analyseur auquel on puisse se fier pour de telles études. Théoriquement, on pourrait coupler la stroboscopie avec un circuit intégré de télévision, lui-même relié à un système d'enregis-

trement magnétoscopique ainsi serait réalisée une méthode complètement objective. Mais, d'une part, il s'agit d'une installation très coûteuse et complexe où intervient, en particulier, la qualité du magnétoscope et, par ailleurs, le temps de rémanence du tube de télévision gène considérablement la stabilité de l'image obtenue. De sorte que nous avons abandonné ces essais. Les difficultés que nous avons rencontrées expliquent que la méthode stroboscopique n'ait pas répondu aux espoirs des chercheurs et que les chiffres qui furent publiés doivent être considérés comme sujets à révision.

LA MICROCINÉMATOGRAPHIE

La cinématographie en prises de vues accélérées est une méthode qui a l'avantage de fournir un enregistrement sur film que l'on peut étudier à loisir et de façon objective, par les méthodes graphiques et chronométriques que nous précisons dans le film réalisé en utilisant l'appareillage suivant: *a*) une caméra (Eclair Coutant) à moteur régulé, fournissant un enregistrement cinématographique à 200 images/seconde, avec une précision très satisfaisante (marge d'erreur de l'ordre de ±1%); *b*) un microscope Zeiss à contraste de phase, type tovar (optique 100×1,5×8, 40×21×8 grossissements suffisants pour individualiser soit les cils, soit une touffe de cils synchrones).

Les principaux problèmes qui se posent sont ceux:

1. de l'éclairage;
2. de l'élimination des vibrations;
3. de la rapidité de mise au point et d'enregistrement cinématographique.

(1) Pour pouvoir filmer sous des grossissements de 600 à 1100 diamètres, environ, à une vitesse de 200 images seconde, il est nécessaire de disposer à la fois, d'une source lumineuse puissante et de films très rapides. Nous avons utilisé une lampe à arc au xénon, munie de filtres anti-caloriques et un film du type 4 X Kodak négatif noir et blanc.

(2) La puissance du moteur et la rapidité du déroulement provoquant des vibrations considérables, il faut, d'une part, séparer le système optique du système cinématographique et, d'autre part, disposer pour fixer la caméra d'un bâti, du type portique, en acier scellé au mur et non solidaire de la table de prise de vue.

(3) La rapidité de la mise au point est très importante, car quelles que soient les précautions prises pour assurer la survie des fragments d'épithéliums étudiés (qui sont celles que nous avons décrites au chapitre de l'observation microscopique) il est certain que les cellules ciliées sont placées en des conditions de survie non physiologiques et qu'il est capital qu'entre la dissection et l'enregistrement il ne s'écoule pas plus de 2 à 3 minutes. Or la caméra utilisée ne disposant d'une visée réflexe que lorsqu'on enlevait le magasin de film — d'où l'impossibilité d'enregistrer rapidement et de suivre optiquement la préparation lors du tournage. Nous avons résolu cette difficulté en incorporant au circuit optique une caméra de télévision qui, après réglage, permet de mettre au point sur l'écran et de filmer en suivant l'image formée sur celui-ci. La quantité de lumière dont nous disposions a autorisé ce dispositif.

Lorsqu'on adopte l'enregistrement cinématographique, deux méthodes de mesure dont les résultats doivent se recouper à 10% près, peuvent être utilisées.

La méthode graphique

Il est possible de projeter sur l'écran le film image par image et de repérer graphiquement en l'entourant d'un trait de crayon, la projection d'un cil donné, dans une position caractéristique. On compte, alors, le nombre d'images nécessaires pour qu'il retrouve cette même position et ce nombre d'images définit le temps d'un cycle du mouvement puisqu'il détermine les passages par une même position. Dès lors, un calcul très simple permet d'établir le nombre de cycles par minute.

La méthode chronométrique

A de tels grossissements et en projetant le film

à 24 images/seconde, on peut très générale ment suivre à l'œil le battement et compter le nombre de cycles décrits par le cil ou une touffe de cils en dix secondes. Ici, encore, un calcul très simple permet de chiffrer ces données. Nous avons appliqué l enregistrement cinématographique à l'étude du rythme ciliaire d'un certain nombre de ciliés marins et de ver tébrés. Ce sont

	Cycles par minute
Mytilus edulis (cils latéro-frontaux de l'épithélium branchial)	1000 à 1100 c/m
Patella vulgata (cils branchiaux)	1100 à 1150 c/m
Acanthochiton (cils de l'épithélium intestinal)	1000 à 1100 c/m
Botryllus schlosseri (épithélium branchial) On doit noter ici la très grande fragilité de ces structures ciliées qui, mises dans les meilleures conditions expérimentales, ne battent guère plus de 15 à 20 minutes. Il est donc très probable que cette valeur soit inférieure à la réalité.	700 à 850 c/m
Asterias rubens (estomac)	1000 à 1050 c/m
Sipunculus nudus (épithélium intestinal)	900 à 950 c/m
Rana esculenta (pharynx)	750 à 800 c/m
Cavia (trachée)	650 à 700 c/m
Gallus (trachée)	600 à 650 c/m

Critique de la méthode

Il convient de noter (et c est un excellent exemple de certaines limitations afférentes à la méthode cinématographique) que nous n avons pas pu mesurer le rythme des battements ciliaires de l'épithélium des parois septales chez *Anemonia sulcata* les cils sont, à la fois, trop nombreux, trop fins et ils offrent un battement trop asynchrone pour qu on puisse en individualiser un seul ou même une touffe. Il n en demeure pas moins que des cas semblables sont certainement rares.

Les seuls autres inconvénients majeurs de la méthode sont d'ordre matériel. Le coût de l'appareillage et de son installation est élevé et la quantité de film utilisé est, évidemment, considérable. C'est une méthode dispendieuse qui ne peut être considérée comme méthode de routine. Mais sur le plan expérimental, les avantages sont considérables, car les cils sont filmés à des grossissements qui autorisent leur étude individuelle. Par ailleurs, c est une mé thode objective qui fournit des documents de travail susceptibles d'être étudiés à loisir et, éventuellement, par plusieurs chercheurs, afin de diminuer le risque inhérent à toute inter prétation subjective

Une critique a été faite par Dalhamn & Rylander (1962) qui signalent que, pour une ca dence donnée de prise de vue la précision di minue en fonction directe de la rapidité du rythme ciliaire. Le fait est exact, mais seule ment pour des fréquences de prise de vue ne dépassant pas 100 images par seconde La fré quence de 200 images par seconde ne justifie pas la critique, le nombre maximum de cycles par minute ne dépassant pas 1100 Certes, une plus grande précision encore pourrait être apportée en utilisant des caméras fonctionnant entre 400 et 600 images/seconde, mais il est douteux qu on puisse disposer d'un éclairage suffisant en ces conditions.

LA PHOTO-OSCILLOGRAPHIE

C'est la plus récente en date des méthodes d'étude du mouvement ciliaire et de ses rythmes, puisqu elle fut proposée par Dalhamn et Rylander en 1962 Ces auteurs partent de la notion que la surface du mucus qui recouvre toutes les surfaces ciliées est soumise à des ondulations synchrones du mouvement ciliaire et donc à des variations d'éclairement en transformant le phénomène lumineux en phénomène électrique, de même fréquence, on peut obtenir un enregistrement parfaitement objectif du rythme ciliaire

Guillerm et Badré (1963) ont, depuis, apporté des améliorations techniques. Ces auteurs utilisent le dispositif expérimental suivant un système optique qui permet un grossissement de 40 à 80 diamètres, composé par un objectif à faible puissance et un oculaire puissant, de manière à allonger la distance focale et, du même coup, la hauteur utilisable

pour le matériel. Un optique latéral est branché sur l'appareillage et dérive 20% environ de la lumière totale La source lumineuse est mobile, afin de pouvoir en tâtonnant, trouver le meilleur angle d'éclairement. Il est constitué par une source dont l'intensité doit être fixe c est un point capital. Ils ont utilisé une lampe à incandescence de 50 watts, sous courant continu de 8 volts, refroidie par un courant d'air En outre, afin de réduire le rayonnement calorique au minimum, un double écran, constitué par un bac contenant une solution saturée de sulfate de zinc et un filtre interférentiel à infra-rouge, a été interposé entre le source lumineuse et le système optique Malgré cette précaution, l élévation de chaleur est notable et peut dépasser 35 si la mesure est prolongée La cellule photo-électrique utilisée était une cellule au cadmium dont la surface sensible était de 2,5 mm² et qui réagit à toute variation d'éclairement par une variation de voltage. Les limitations de l'utilisation de ces cellules sont les suivantes du point de vue physique

(*a*) maximum de sensibilité entre 650 et 700 mμ c est à dire dans le rouge et donc dans longueur d'onde la plus calorigène L obli- d'arrêter les infra-rouges et une partie rouge visible en raison de ce dernier effet, une certaine perte de sensibilité de la cellule

(*b*) la nécessité d'avoir un éclairage parfaitement constant;

(*c*) la nécessité d offrir à la cellule photo-électrique l'image la plus contrastée possible, car sa réponse est déterminée par le rapport du signal sur le bruit de fond

Les fluctuations de voltage sont injectées à un amplificateur et transmises à un enregistreur muni d'un stylet qui fournit directement un oscillogramme à partir duquel, connaissant la vitesse de déroulement en fonction du temps, on peut compter la fréquence des fluctuations lumineuses.

Telle que nous venons de la décrire, cette méthode soulève un certain nombre de critique

(1) Utilisant un grossissement de 80 diamètres, elle est évidemment incapable de mettre en évidence les fluctuations lumineuses produites par un seul cil.

(2) Utilisant une cellule d une surface considérable (2,5 mm²) à l échelle cellulaire, elle enregistre les variations, non pas d'une touffe ciliaire, mais de centaines d'entre elles de façon globale. Or on sait que le mouvement ciliaire, s'il peut être synchrone en ce qui concerne une touffe ciliaire implantée sur une seule cellule (le cas est très net chez les cellules ciliées de la trachée du coq par exemple) est toujours asynchrone (ou métachrone, ce qui revient au même ici lorsqu une telle surface d'épithélium ciliée est en cause. Ce que l'on mesure est la résultante globale sur une grande surface de mucus de ces milliers de mouvements et non le rythme ciliaire proprement dit, même si cette résultante est voisine de ce rythme, on ne peut pas vérifier immédiatement ce fait capital.

(3) Les conditions physiologiques de l'expérimentation sont également discutables. On sait que la viscosité du mucus est un paramètre très important de la rapidité du rythme Or au niveau d'une trachée largement ouverte et chauffée par les rayons lumineux il est certain que la mince pellicule liquide de mucus doit subir une évaporation importante qui augmente considérablement sa viscosité.

(4) Enfin, et c est peut-être là notre plus grave critique, la méthode telle qu elle est décrite ne peut pas être appliquée à tous les épithéliums ciliés, puisqu'il est nécessaire que ceux-ci offrent une surface recouverte de mucus En d'autres termes, elle élimine de l'étude les animaux marins et les protistes qui sont de beaucoup les plus nombreux et les plus variés parmi les ciliés.

Cependant, la méthode a le mérite d'être objective et, à ce titre, nous avons repris son étude dans le laboratoire de M le Professeur Bessis afin de tenter d'améliorer ses performances sur le plan physique pour l'amener à enregistrer les fluctuations lumineuses produites par un seul cil, ou, à défaut, celles pro-

duites par une touffe ciliée unicellulaire, et cela aussi bien chez les animaux marins que chez ceux qui mènent une vie aérienne

LA MICROPHOTO-OSCILLOGRAPHIE

L appareillage que nous avons utilisé a été mis au point en vue de recherches de microspectrophotométrie, mais il peut s adapter sans modifications notables, aux exigences de la pré sente expérience

Le principe de la microphoto-oscillographie consiste à former l'image très agrandie d'un cil sur un petit diaphragme d un diamètre sensiblement égal à la dimension transversale de l'image du cil. Dans ces conditions, lorsque le cil effectue un battement, il module la lu mière traversant le diaphragme et ces modulations reproduisent évidemment le rythme du mouvement ciliaire La seule difficulté d'inter prétation rencontrée lors des expériences tient au fait qu'il est possible, dans les conditions expérimentales ci-dessus définies, d'enregistrer le premier harmonique de la fréquence fondamentale du battement ciliaire. En effet, si un cil est parfaitement centré sur le diaphragme, il passe deux fois devant ce dernier fournissant ainsi deux déflexions pour un seul cycle un déplacement minime de la préparation per met de la placer de façon à ce que le diaphragme corresponde à une des positions ex ternes du cycle et l'on obtient ainsi le rythme ciliaire fondamental.

Dans le détail, l'appareillage était constitué par un microscope (Wild M 20) équipé d'un optique à contraste de phase, objectif à immer sion × 100 La source lumineuse est constituée par une lampe à vapeur d'iode, la lumière est filtrée par un filtre interférentiel MTO et divisée en deux faisceaux pour permettre simultanément l'observation et la mesure

Avec un objectif à immersion × 100 le grossissement de l'image projetée sur le plan du diaphragme est de 1200 diamètres. Dans ces conditions, un diaphragme de un millimètre de diamètre offre dans le plan de l objet une image de 0 8 μm valeur qui correspond sensiblement au diamètre d'un cil.

La lumière qui traverse le diaphragme est mesurée par un photomultiplicateur (Radiotechnique A V P 150) Le signal électrique sortant du photomultiplicateur est amplifié puis filtré au moyen d'un filtre électronique passe-bande dont le rôle est d atténuer d'une part, le bruit de haute fréquence (provenant essentiellement du photo-multiplicateur) et, d'autre part, le bruit de basse fréquence (produit essentiellement par les légers déplacements de la préparation) Le signal qui représente la modulation du flux lumineux par la structure ciliée étudiée est alors envoyé sur un enregistreur potentiométrique (Texas Instruments Servo Riter II) qui est capable de suivre sans atténuation exagérée des fréquences de 10 à 15 Hz, soit 600 à 900 oscillations minute On peut, d'ailleurs, également transmettre le signal à un oscilloscope, à partir duquel on peut obtenir avec une fréquence de balayage convenablement choisie un enregistrement photographique.

Enregistrement thermique

Lié de façon étroite à ce problème de l'en registrement des battements ciliaires apparait celui de la température exacte à laquelle est soumise la préparation au moment ou s ef fectuent les mesures. Il est, en effet, d une im portance capitale de connaître exactement, lorsqu on étudie tout phénomène biologique au microscope et particulièrement un mécanisme tel que le mouvement ciliaire qui met en jeu une importante consommation d'énergie, la température exacte à laquelle est soumise la préparation Et lorsque nous disons prépara tion, cela signifie la température de la zone lumineuse qui sert en microscopie optique à l'observation tissulaire (éventuellement, cette zone peut être rendue notablement plus pe tite que le champ du microscope) Afin de résoudre ce problème, qui d'ailleurs revêt une valeur très générale en physiologie cellulaire, nous avons pu utiliser un microthermocouple (Cu-Constantan) dont la soudure a une dimen-

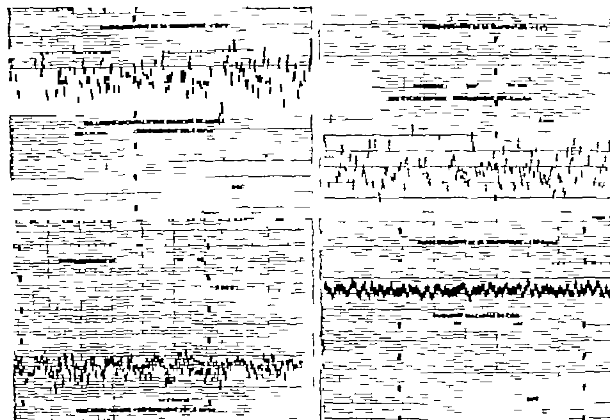


Fig 1

lon de 50 μm Ce thermocouple est fixé sur lame d observation microscopique et ses faibles dimensions permettent la mesure é la température dans le champ d'observation. La sensibilité de ce microcouple est grande, puisqu elle fournit aisément le cinquième de degré Dans ces conditions, nos mesures des rythmes du battement ciliaire ont pu être effectuées à température constante et contrôlée, la température étant enregistrée sur la même bande de papier que le tracé représentant le battement ciliaire Nous avons pu ainsi constater qu avec l éclairage, relativement faible que nous utilisions, grâce à la sensibilité du photomultiplicateur et à l'emploi de filtre anticalorique, la température de la préparation enregistrée au niveau de son éclairement ne s élève pratiquement pas par rapport à l ambiance (en pratique de 1 4 à 1 2 degré centigrade, ce qui est tout à fait négligeable). Par contre, nous avons pu constater qu en utilisant un éclairage mal étudié (filtrage insuffisant, en particulier) on enregistre des élévations de température qui peuvent atteindre 15 Nous avons effectué nos enregistrements à deux températures. 24–25 d'une part, et 5–6 d autre part. Ces dernières sont obtenues, non pas en refroidissant la platine du microscope, mais en travaillant dans une chambre froide réglée à 4

Nous avons étudié par microphoto-oscillographie la ciliature de cobaye (*Cavia*) de coq (*Gallus*) d'étoile de mer (*Asterias rubens*) et de moule (*Mytilus edulis*) Les résultats obtenus furent les suivants

pour le cobaye	485 à 570 cycles par minute
pour le coq	650 à 700 cycles par minute
pour le moule	500 à 550 cycles par minute
pour l'étoile de mer	600 à 650 cycles par minute

et en ces deux derniers cas, à la température de 5° aussi bien qu'à 24°

Si l on compare ces chiffres à ceux fournis par l enregistrement cinématographique en prise de vues accélérées, il ressort que les cyc

les de battement trouvés par les deux méthodes concordent de façon assez satisfaisante en ce qui concerne les cycles des animaux homéothermes. Les différences, de l'ordre de 10% trouvées peuvent tenir au fait que l'enregistrement cinématographique fut certainement effectué à température plus élevée, de l'ordre de 34 *sans que nous puissions le préciser aussi exactement que dans ce dernier cas.*

Pour les animaux marins, par contre, les différences sont de l'ordre de 40% et ne peuvent s expliquer que par la différence des conditions expérimentales. Le rythme du battement ciliaire des animaux marins (*Asterias rubens* et *Mytilus edulis*) fut, en effet, étudié alors qu'ils avaient été conservés 48 à 72 heures en eau de mer dans des conditions peu favorables, en *particulier sous l'angle de l'oxygénation du mi*lieu, et il nous semble probable que ce facteur ait joué un rôle important dans la diminution du rythme ciliaire mesuré

CONCLUSION

De toutes les nombreuses méthodes que nous avons étudiées (et pour certaines d'entre elles expérimentées) et qui toutes, *depuis près d'un* siècle, s efforcent de résoudre le problème de la mesure des rythmes du battement ciliaire *il nous paraît que seules deux d'entre elles* la microcinématographie, en prise de vues accélérées, et la microphoto-oscillographie peuvent prétendre fournir une solution à ce problème et ceci au niveau de tous les épithéliums ciliés.

En fait, ces deux méthodes peuvent et doivent se combiner Le cinéma offre, grâce aux deux méthodes, graphique et chronométrique que nous avons précédemment exposées, la possibilité d'une mesure exacte, mais ceci sous la condition que l'on puisse individualiser un cil sur l écran de projection. C'est là une limitation dont il ne faut pas négliger l'importance Par contre la microphoto-oscillographie peut, par les dimensions utiles du *diaphragme qui* constitue l'élément essentiel de *sélection de* l'image et de la modulation de la lumière, éviter cet écueil. En outre, les résultats de cette dernière méthode sont obtenus de façon plus immédiate et autorisent des expérimentations pharmacodynamiques plus aisément réalisables. C'est dans cet esprit que nous pouvons affirmer que la combinaison de ces deux méthodes doit pouvoir résoudre les problèmes *posés par l'étude des rythmes du mouvement* ciliaire.

SUMMARY

After emphasizing the importance of ciliary beating both as pharmacodynamic test and as a biological index of the cellular state, the uthors report their experiences after studying wide range of ciliary structures both marine and land-l ing. These experiences used three objective methods: ( ) the stroboscopic; (*b*) *the* cinematographic ( ) the microphoto-oscillographic.

The stroboscopic method, to be really object ought to couple the stroboscope with an integrated television set feeding magnetoscope for recording Otherwise the frequences are almost unbearable for *the eye of the experimentator*

The cinematographic method using a camera with a speed of recording of 200 pictures minute gives results that can be analysed ery precisely by both graphic and chronometric method. But to use these methods, the individualisation of one cilia or at least bunch of synchronous ones must be possible on the screen.

The microphoto-oscillography is an improvement of the method proposed in 1962 by Dalhamn and Rylander The principle of the method w have used is to form the enlarged picture of one cilia on little diaphragm that has diameter of about the same size as the width of the enlarged cilia. Under these conditions each ciliary beating modulate the light that goes through the diaphragm and photomultiplicator and these modulations follow the rythm of the beating f this individual cilia (technical details re described in the text). During these experiments, the temperature was precisely registered by micro-thermocouple, no more than 50 µm *large at its point*, that was fi ed on the microscopic slide. During the experiment, the temperature f the very spot where the cells were beating could be registered with an accuracy f 1 5 f degree.

A conclusion there is no doubt that combining the two methods, the cinematographic high speed recording and the microphoto-oscillography yield objective result that are certainly very near the physiological normal beating f the different ciliary struc*ture. As far as routine examination is concerned*, as for instance when pharmacodynamic studies are concerned, the microphoto-oscillography appears as the most convenient method.

Acta

ZUSAMMENFASSUNG

Nachdem die Bedeutung der objektiven Studie der Ciliarbewegung, die gleichzeitig einen pharmakodynamischen Test und einen genauen Index des physiologischen Zustandes der Ciliarzellen darstellt, hervorgehoben wurde, berichten die Verfasser über ihre Erfahrungen mit drei verschiedenen Untersuchungsmethoden: (a) die Stroboskope, (b) die kinematographische Aufzeichnung mit beschleunigter Filmaufnahme und (c) die Mikrophoto-Oszillographie.

Diese letzte Methode ist eine Verbesserung der photo-oszillographischen Methode, die 1962 von Dalhamn und Rylander vorgeschlagen wurde.

Die Bewegungen einer einzigen Cilie sind imstande den Lichtstrom zu modulieren und erlauben eine entweder graphische oder photographische Registrierung. Ein Mikroregistrierapparat für die Temperatur ist diesem System angeschlossen.

Die durch diese verschiedenen Methoden gewonnenen Ergebnisse zahlreicher Untersuchungen von Flimmerepithel werden erläutert und verglichen.

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MIDDLE EAR IMPEDANCE MEASUREMENT

Acoustic and Electroacoustic Comparisons

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Abstract. Acoustic and electroacoustic methods of measuring normal middle ear impedance were compared and evaluated. It was concluded that normal subjects yield similar impedance results in the areas wher comparisons are possible. There are advantages and disadvantages with either technique, and the method of choice depends upon the type of impedance information desired.

The diagnostic value of human middle ear impedance measurement has been demonstrated by various investigators over the past twenty years, but prototype measuring devices have been more suited to the experimental laboratory than the clinic (Metz, 1946, 1951 Kristensen & Jepsen, 1952 Zwislocki, 1957 *a*, 1957 *b* 1961 Terkildsen & Nielsen, 1960). Recently two instruments have been developed specifically for the clinical assessment of middle ear pathology by the measurement of middle ear impedance, one by an acoustic impedance matching method, the other by elec troacoustic calibration. These instruments are the Zwislocki Acoustic Bridge and the Madsen Electroacoustic Impedance Bridge. The two measurement systems involved are quite dif ferent, as are also the type and form of data obtained with each. Furthermore normative absolute impedance data, while becoming more available with the Zwislocki bridge are still unavailable for the Madsen bridge The present study while normative in nature mainly an attempt to find comparable between the two systems and asses. [illegible] gree of relationship

The Zwislocki bridge is a return tc original intent of Metz (1946) [illegible] h s atte to measure the absolute impedance middle ear mechanism. Th. bridge is c so that its matching impedance measure sistance and compliance These are mea separately though simultaneously Th. sistance dial is calibrated in terms of arbi[illegible] units and the compliance dial in terms of of equivalent volumes of air From these [illegible] ues, physical descriptions of resistance, compliance, reactance and absolute impedance may be mathematically derived. Absolute impedance measurements may be made at any fre quency with this instrument, though those be low 1.5 Hz are considered the most diagnostic. Acoustic reflex testing or measurement of the sound level at which the acoustic reflex is elicited may also be performed at any test frequency or with any noise source, through the detection of relative impedance changes.

The Madsen Electroacoustic Impedance Bridge measures absolute impedance only at 220 Hz, the frequency of its calibration tone. Compliance (in terms of cc of equivalent v[illegible]

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umes of air) and the absolute impedance (in Acoustic Ohms) may be computed from scale readings at this frequency As with the Zwislocki Acoustic Bridge, the sound level at which the acoustic reflex is elicited may be measured at any test frequency or with any noise source through the detection of relative impedance changes. In addition, positive or negative air pressures in the middle ear cavity may be measured When coupled to a level recorder changes in impedance due to induced pressure changes in the external auditory canal may b• recorded and measured (Lidén, 1969)

SUBJECTS AND EQUIPMENT

Twenty-one young adult subjects were tested, 16 female and 5 male, whose hearing thresholds were no poorer than 10 dB (re ISO 1964 norms) at the frequencies 250 500 1 000 2 000 4 000 and 6 000 Hz. The subjects had no known middle ear pathology or family history of hearing loss As an additional indicator of middle ear normalcy presence of a positive acoustic reflex was required bilaterally of all subjects

A Zwislocki Acoustic Bridge, Model 3 Grason–Stadler Co) was used, with the probe supplied by a Békésy audiometer (Grason-Stadler E-800) operated at discrete frequencies. The comparison instrument was the Madsen Electroacoustic Impedance Bridge, Model ZO-70 (Madsen Electronics) Pure tone stimuli for acoustic reflex testing were supplied by a Beltone Model 15 C audiometer equipped with TDH 39 earphones The same audiometer and earphone were used with both the Zwislocki and Madsen instruments for reflex testing.

PROCEDURE

Both ears of each subject were tested, giving a total N of 42 ears. Subjects were first tested with the Madsen instrument. The test sequence for each subject was. measurement of middle ear pressure, absolute impedance at 220 Hz, and acoustic reflex testing at 500 1 000 and 4 000 Hz. Immediately afterwards, the same subject was tested with the Zwislocki instrument, the sequence being measurement of external auditory canal volume followed by absolute impedance measurements at 125 220, 250 750 1 000 and 1 500 Hz. Complete acoustic reflex testing was performed at a later date on five subjects (10 ears) with the Zwislocki bridge at the same frequencies previously tested with the Madsen bridge (500 1 000 and 4 000 Hz)

All measurements with both bridges were made by two of the authors (GRH and KSB) who had had extensive experience with that instrument.

RESULTS AND DISCUSSION

Middle ear pressure

Measurement of middle ear pressure is possible with the Madsen instrument but not with the Zwislocki Table I shows results obtained with 40 ears (One subject was eliminated from the Madsen data because of difficulties in obtaining an airtight seal in the external canal.) Madsen suggests that negative pressures of 25 to 50 mm (H_2O) are not particularly significant. All subjects tested were well within this range Since no comparison between bridges is possible with pressure measurements, the chief value of the data was to help confirm the middle ear normalcy of the test group

Acoustic reflex

Acoustic reflex levels, indicated by monitoring relative impedance changes, may be measured with both bridges. Detecting these changes (by ear) with the Zwislocki bridge is a more difficult task than with the Madsen bridge, which displays them visually The greater variability of scores obtained with the Zwislocki bridge in the present study may reflect this difficulty While attachments are available for the Zwislocki bridge which enable the operator to monitor impedance changes through a sound level meter the technique is cumbersome and difficult.

Table I. *Average middle ear pressure values obtained with the Madsen bridge*

	Middle ear pressure (mm H_2O)
Mean	−2.31
S.D.	12.15
S.E.	1.90

(N 40)

Acoustic reflex results obtained with the two bridges are compared in Table II. Analysis of variance yielded no F-ratios significant at the 0.05 level of confidence. Pearson r's at each frequency were high but negative. No explanation for this is readily apparent. Mean differences between reflex levels obtained with the two techniques were small, the largest (3.5 dB) occurred at 1 000 Hz. Results with the Madsen bridge were consistently less variable than those obtained with the Zwislocki bridge, as noted. For the purpose of studying the acoustic reflex through relative impedance changes, the Madsen technique seems superior. The procedure is simple, efficient and objective.

Acoustic impedance

Table III shows impedance and compliance data obtained with the Madsen bridge at 220 Hz. Table IV shows resistance and compliance data obtained with the Zwislocki bridge at the seven frequencies tested. The results of Table IV are in close agreement with normal impedance data published by Feldman (1967) Zwislocki (1968) and Burke et al. (1970).

To make a direct comparison between the Madsen and Zwislocki bridge data, the Zwislocki resistance and compliance measurements at 2?0 Hz were converted to impedance.[1]

This comparison is shown in Table V. The mean normal impedances obtained with the two instruments are very close and the correlation between scores (Pearson r) of 0.81 is very high. The relationship between scores is illustrated in Fig. 1. However, an analysis of variance (treatments × subjects design) yields a significant F-ratio at the 0.05 level indicating that a difference of this magnitude (162 ohms) between means would occur by chance less than five times in one hundred. A test for homogeneity of related variances was then performed. The resulting t of 1.22 was not significant at the 0.05 level, indicating a probable normality of the data. (A possible reason for the significant mean difference is discussed below.)

Acoustic compliance (in cc of equivalent volume) results with the two instruments are also quite close (Table VI). Mean volume dif

[1] Individual scores were first converted from compliance (in cc of equiv. vol.) to reactance by the formula:

$$\text{Reactance} = \frac{1.43 \quad 10^{6}}{6.28 \quad \text{frequency} \quad \text{equiv.} \quad \text{vol. (cc)}}$$

Impedance was then calculated from resistance and reactance by the formula.

$$\text{Impedance} = \sqrt{\text{Resistance}^2 + \text{Reactance}^2}$$

Table II. *Stimulus levels required to elicit the acoustic reflex. Measurements with both Madsen and Zwislocki bridges*

	Stimulus level (in dB re ISO 1964)					
	500 Hz		1 000 Hz		4 000 H	
	Madsen	Zwislocki	Madsen	Zwislocki	Madsen	[illegible]
Mean	94.0	92.0	92.5	96.0	[illegible]	[illegible]
S.D.	5.2	6.8	2.6	4.6	[illegible]	[illegible]
S.E.	1.6	2.1	0.8	1.4	[illegible]	[illegible]
Pearson	65		−46		[illegible]	

(N 10)

Table III *Acoustic impedance and compliance measurements with the Madsen bridge*

	220 Hz	
	Impedance (Ac. Ohms)	Compliance (cc of Equiv Vol.)
Mea	1 650.	748
S.D	738 7	.332
S.E.	115 4	.052

(*N* = 40)

Table V *Acoustic impedance Comparison of results obtained with the Madsen and Zwislocki bridges at 220 Hz*

	Impedance (Acoustic Ohms)			
	Mean	S.D	S.E.	Pearson
Madsen	1 650	738.7	115 4	
Zwislocki	1 812	677 6	105.8	.81

(*N* = 40)

ferences are small and the correlation between measures is again high and positive (0 72) The standard deviation and standard error obtained with the Zwislocki bridge are smaller than those obtained with the Madsen bridge (These findings are also true with the impedance measures as seen in Table V) Analysis of variance yielded a significant *F*-ratio only at the 0 10 level of confidence indicating that mean differences of that magnitude could be expected by chance ten times in one hundred. However the test for homogeneity of related variances yielded a significant *t* at the 0 05 level. Compliance variances obtained with the two techniques were therefore significantly different, making dubious the application of u statistics to this measure, since he ity of variance is often accompanied by n-normality of the distribution. Feldman (1967) had noted earlier that the slightly skewed distribution of scores he obtained with the compliance measure of the Zwislocki bridge made doubtful the proper application of such measures as the standard deviation. However the Norton study described by Lindquist (1956) showed that with the *F*-test of analysis of variance, lack of symmetry in the distribution is not too critical, as long as the curve is not too flat or peaked. Furthermore, heterogeneity of variance must be quite extreme to be of any serious consequence

It should be noted at this point that no correction factor has been added to the compliance data reported here. Feldman & Zwislocki (1965) added 0 1 cc to their compliance data and Feldman (1967) recommended adding 0 05 cc at all frequencies in order to allow for a residual volume apparently built into the Zwislocki bridge. This residual volume causes the bridge to consistently yield lower compliances than those obtained by electroacoustic methods. Referring to Table VI, an addition of 0 05 cc to the Zwislocki bridge data would increase the compliance mean to 0 735 cc. This value is extremely close to the Madsen compliance mean of 0 748 cc. While adding

Table IV *Acoustic resistance and compliance measurements with the Zwislocki bridge*

	125 Hz	220 Hz	250 Hz	500 Hz	750 Hz	1 000 Hz	1 500 Hz
	Resistance (Acoustic Ohms)						
Mean	699	588	541	439	395	384	325
S.D	229 6	200.5	203.1	139.5	93 7	98.2	99.8
S.E.	35 4	31.3	31.3	21.5	14.5	15.2	15 4
	Compliance (cc of Equiv Vol)						
Mean	649	685	708	.883	1 113	1.331	1.355
S.D	.214	.239	.247	.391	.580	.590	.520
S.E.	033	038	038	060	089	091	.080

(*N* 42)

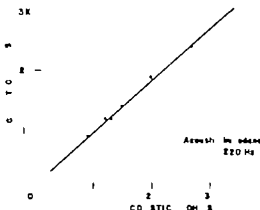


Fig. 1. Correlation between Madsen and Zwislocki impedance at 220 Hz, with line of regression. ($y' = 0.829 + 0.873x$.) *X*-axis represents Madsen bridge data, *Y*-axis, Zwislocki bridge data.

a constant would not affect the Zwislocki variance or the resulting variance differences between instruments, it would have the effect of reducing both the Zwislocki mean reactance and impedance. Therefore, the mean impedance difference (162 ohms, as noted earlier) obtained between the two bridges (Table V) would be reduced. This indicates that the two measurement systems (acoustic and electroacoustic) do indeed yield similar impedance results, and that these results are highly correlated. Compliance results are also similar and highly correlated, but statistical normality of the data may be questioned. It is regrettable that absolute impedance comparisons between the two bridges are possible at only one frequency (220 Hz).

SUMMARY AND CONCLUSIONS

The above data indicate that with a sample of young normal adult middle ears the Zwislocki and Madsen bridges yield similar impedance results where comparisons are possible. This finding is in agreement with, and extends the conclusion of Terkildsen & Nielsen (1960) that both the mechanical (acoustic) and electroacoustic methods are concerned with variations of the same acoustic properties of the ear. However, neither instrument can be com-

Table VI. *Acoustic compliance. Comparison of results obtained with the two methods at 220 H*

	Compliance (cc of Equiv. Vol.)			
	Mean	S.D.	S.E.	Pearson
Madsen	.748	.332	.052	
Zwislocki	.655	.239	.038	.77

($N = 40$)

pletely evaluated on the basis of the areas common to both. While the aim of each is to explicate middle ear impedance and predict middle ear pathology, the approach and major emphasis of each bridge is different. The Zwislocki bridge is most capable of measuring absolute impedance over a wide frequency range. Relative impedance changes are measured with more difficulty. The Madsen bridge yields little absolute impedance data, but is more capable when measuring relative impedance changes and middle ear pressure.

Determinations of the sound level necessary to elicit the acoustic reflex may be made with either bridge at any frequency desired. The Madsen electroacoustic technique seems to be more objective and less variable than the Zwislocki technique in this area. The acoustic reflex has been utilized in a variety of tests of recruitment, pseudohypacusis, facial nerve paralysis, and the separation of conductive from sensorineural pathology. However, in many types of conductive pathology its information value is limited to a determination of whether the reflex is present or absent. This "yes or no" response may be detected with equal ease by either type of bridge.

Measurement of middle ear air pressure may be performed with the Madsen bridge, but not with the Zwislocki. Measurement of changes in middle ear pressure has been related to pathologies such as occluded or patulous Eustachian tube, secretory otitis media, discontinuity of the ossicular chain (Madsen), adhesions or effusion in the middle ear, and supermobile tympanic membrane (Lidén, 1969).

Both methods (acoustic and electroacoustic)

of studying middle ear impedance may yield valuable experimental and clinical data. Both instruments evaluated (Zwislocki and Madsen) represent a great improvement over the Schuster type bridge originally used by Metz (1946) Calibration, measurement, and data re duction have been simplified enough to war rant clinical use. Each bridge has its strong and weak areas which tend to complement rather than replace the other Further study is needed of pathologic ears with both methods

ZUSAMMENFASSUNG

Eine akustische und eine elektro-akustische Messmethode zur Ermittlung des normalen Scheinwider standes (Impedanz) im Mittelohr wurden verglichen und ausgewertet. Die Schlussfolgerung war dass sich bei normalen Versuchspersonen in Bereichen, wo eine Vergleichsmöglichkeit besteht, bei beiden Verfahren ähnliche Werte ergaben. Jedes der beiden Verfahren hat Vorteile und Nachteile die Wahl der Methode hängt davon ab welche Impedanzwerte man ermit teln möchte

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STEIGBÜGELMISSBILDUNGEN

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Abstract. Nach einer kurzen Darstellung der Stapesentwicklung wird über 8 selbst beobachtete Fälle berichtet, die in einem Krankengut von etwa 1000 Schalleitungsschwerhörigkeiten bei reizlosem Mittelohr gefunden wurden. Die mögliche Genese der einzelnen Mißbildungsformen wird diskutiert. Im gleichen Krankengut fanden wir in 0,5% Aplasien des M. stapedius. Als Folgerung aus den funktionellen Ergebnissen, die operativ erzielt wurden, läßt sich feststellen, daß mit den heute bekannten Methoden der Stapeschirurgie in den meisten Fällen eine wesentliche Besserung des Hörvermögens zu erreichen ist.

Schwere, das Hörvermögen beeinträchtigende Steigbügelmißbildungen werden häufig zusammen mit großen Mißbildungssyndromen des Schädels gefunden. Bei der Dysostosis mandibulo-facialis (Franceschetti-Zwahlen- oder Treacher-Collins-Syndrom) wurden Steigbügelmißbildungen z. B. von Altmann (1933, 1957, 1965), Plester (1961), Herberts (1962) und Schreiner (1962) beschrieben. Auch bei der Dysostosis cleido-cranialis (Morbus Crouzon) sowie bei den lebensunfähigen Dysmorphien wie der *Otocephalie, der Arrencephalie und den* Monstrenbildungen werden sie in der Regel gefunden (Altmann, 1957).

Bei der Talidomid-Embryopathie stehen Ohrmißbildungen nach den Extremitätendysplasien an zweiter Stelle in der Häufigkeit. Diese Kinder werden in der Regel erst im Schulalter gehörverbessernden Operationen unterzogen (Partsch, 1964). Deshalb liegen bisher nur wenige detaillierte Beschreibungen der Mittelohrbefunde bei diesem Krankheitsbild vor. Da es bei der Talidomid-Embryopathie im Bereich des Schädels aber vor allem zu einer Schädigung des Hyoidbogens kommt, kann mit einer besonderen Häufung von Steigbügelmißbildungen gerechnet werden (Kleinsasser & Schlothane 1965). Nach Pfeiffer & Nessel (1962), Kittel (1963) sowie Miehlke & Partsch (1963) manifestiert sich die teratogenetische Wirkung des Contergans vor allem zwischen der 4. und 7. Schwangerschaftswoche. In diesem Entwicklungsabschnitt liegt auch die „sensible Phase" der Stapesentwicklung.

Häufig werden Steigbügelmißbildungen verschiedenster Art auch bei der sogenannten Atresia auris congenita gefunden. Unter diesem *Oberbegriff werden von den meisten Autoren* alle Grade und Kombinationen von Mißbildungen des äußeren und Mittelohres zusammengefaßt. Auch isolierte Fehlbildungen der *Gehörknöchelchen hinter einem* intakten Trommelfell — von de Witt (1958) als „Atresia auris minima" bezeichnet — werden diesem Oberbegriff untergeordnet.

Die Beschreibung von Organmißbildungen und die Diskussion ihrer Genese setzen die Kenntnis der normalen Organentwicklung voraus. Der Stapes bildet das Kopplungsglied zwischen dem Schalleitungsapparat und dem Schalltransformationsorgan im Labyrinth. Die innige und funktionsgerechte Verbindung zwischen dem eigentlichen Sinnesorgan und der vorgeschalteten Schalleitungskette wird durch embryonale Verschmelzung von Mesenchym beider Bereiche bei der Stapesentwicklung realisiert. In den ersten Embryonalwochen differenzieren sich zunächst die den Keimling ernährenden Blutgefäße, die primitiven Anlagen der Sinnesorgane sowie das zentrale und periphere Nervensystem. Noch vor der Abzeichnung der Stapesanlage im embryonalen Mesenchym erkennt man bereits beim 4,5 Wochen alten Keimling im Bereich des dorsalen Endes des 2. Branchialbogens die A. stapedia

den N facialis (House & Patterson, 1964). Als Ast der A. hyoidea (2. Kiemenbogenarterie) zieht die A. stapedia dorsal von der ersten Schlundtasche nach cranial in das Mesenchym des 1 Kiemenbogens. Sie versorgt auf dem Höhepunkt ihrer Ausbildung gegen Ende der 6. Woche (Embryo von 16–18 mm Länge) über drei Hauptäste eine Region, die dem Ausbreitungsgebiet der drei Trigeminusäste entspricht. Über sich ausbildende Anastomosen finden Äste dieses Gefäßes Anschluß an die A. carotis externa und ophthalmica Im Verlauf des 3 Embryonalmonats obliteriert dann der aus der A. carotis interna gespeiste und durch das Mittelohr verlaufende Stamm der A. stapedia (House & Patterson, 1964).

Der Facialis, Nerv des 2. Branchialbogens, spielt bei der Separierung des Stapesblastems aus dem Mesenchym des Hyoidbogens nach Hanson, Anson & Bast (1959) eine entscheidende Rolle. Beim 4,5 Wochen alten menschlichen Embryo drückt er in das craniale Blastemende der 2. Visceralspange eine sich allmählich vertiefende Kerbe ein und trennt damit die Stapesanlage vom Blastem des Reichert Knorpels ab. Die Stapesanlage stellt zu dieser Zeit noch eine solide Konzentration von Mesenchymzellen dar die lateral der Otocyste gelegen und von deren mesenchymaler Kapsel deutlich getrennt ist. Der zwischen beiden verlaufende Stamm der A. stapedia wird zunächst hufeisen- dann ringförmig von diesem Blastem umwachsen. Mit 5,5 Wochen kann die Arterie schon tief in die Stapesanlage eingebettet sein, in anderen Fällen jedoch ..ch noch peripher von ihr liegen (Hanson *et al* 1959 Anson *. al.*, 1960). Nach Ende der 6. Woche hat das branchiogene Stapesblastem bereits Ringform. Die Stapesschenkel sind zu dieser Zeit plumpzylindrisch. Aus der noch vorhandenen dünnen Brücke (Inte hyale) zwischen der .. Visceralspange und der Stapesanlage differenzieren sich in .. 7 Woche der M. stapedius und seine Sehne. Zu ..er Zeit gewinnt die wachsende Stapesanlage ..ch ... an das Mesenchym der Labyrinthkapsel. ..urch induktiven Einfluß wandelt sich der mit dem Stapesring verschmelzende Teil der Vestibulumwand zur Lamina stapedialis um. De Knorpel in der Peripherie des ovalen Fusionsgebietes wandelt sich allmählich zum Ringband um So verliert die neugebildete Fußplatte ihren festen Zusammenhang mit der übrigen Ohrkapsel

Etwa in der 19 Embryonalwoche beginnt die Ossifikation der Stapesanlage mit einem Herd in der tympanalen Schicht der Fußplatte Von hier ..s über die Crura fortschreitend erreicht ..e beim 21 Wochen alten Feten das Stapesköpfchen. Der Steigbügel hat zur gleichen Zeit seine endgültige Größe erreicht. Nur die der Lamina stapedialis entsprechende dünne vestibuläre Schicht der Fußplatte bleibt ständig knorpelig. Die Verschmelzungszone der beiden embryonalen Teile bleibt deshalb auch beim Erwachsenen histologisch noch deutlich differenzierbar In der sich anschließenden Entwicklungsperiode werden die ursprünglich hohlen, röhrenförmigen Schenkel ummodelliert. Im Gegensatz zu allen anderen Knochen werden sie bis zur 35 Woche immer dünner Gleichzeitig verlieren sie ihren zentralen Markraum dadurch, daß sich die knöchernen Zylinder nach dem nun nicht mehr runden Intercruralraum zu öffnen und in .. nach ..ußen geschlossene Halbrinnen umwandeln.

Es darf wohl als sicher gelten, daß die charakteristische Ringform des Steigbügels durch die Lagebeziehung des wachsenden Stapesblastems zur A stapedia verursacht wird. Seine endgültige Form muß jedoch Kräften zugeschrieben werden, die dem Blastem des 2. Kiemenbogens immanent sind. Nach Oesterle (1933) haben Druck und Zugkräfte keine Bedeutung als wirksames Moment für den Umbau der Ossikula.

Welche Faktoren sind es nun, die die uns hier interessierenden Fehlbildungen des Stapes verursachen? Die entscheidende Determinationsperiode der mesenchymalen Stapesanlage liegt zwischen der 5 und 18 Woche der embryonalen Entwicklung. Nach Virchow sind isolierte Fehlbildungen, die die Grenzen einer Organanlage nicht überschreiten, endogenen Ursprungs. Eine Reihe von Autoren (Hörbst & Sauser 1937 Marx, 1926) sieht deshalb in einem abnormen Verlauf einem besonders großen Kaliber oder in fehlender Rückbildung der A. stapedia die Ursachen für Fehlbildungen wie die partielle oder totale Agenesie eines oder beider Schenkel oder gar des gesamten Steigbügels Hough (1958) vermutet als Ursache der fehlenden Ringform des Stapes eine mangelnde Differenzierungsfähigkeit des Stapesblastems. Diese Ansicht scheint eine Bestätigung durch die Beobachtung von Hanson et al (1960) zu finden, die bei einem 6,5 Wochen alten (14 mm langen) Embryo neben einer normalen A stapedia einen nur einschenkeligen Steigbügel sahen. Das nicht genügend differenzierungsfähige Stapesblastem vermag sich wahrscheinlich nicht um die A. stapedia zu falten. Das Resultat ist eine Hemmungsmißbildung, die nach Rossberg (1963) durch das Zurückbleiben eines einzelnen Teils einer Organanlage charakterisiert ist.

Eine exogene toxische Schädigung des Blastems muß bei der Talidomid-Embryopathie angenommen werden

Zusammenfassend kann also gesagt werden, daß sowohl vaskuläre Irritationen als auch eine Entwicklungsinsuffizienz des Blastems neben anderen Ursachen verschiedener Art für die verschiedenen Stapesfehlbildungen verantwortlich sein können.

Eigene Beobachtungen

Die persistierende A. stapedia gilt als eine sehr seltene Anomalie. Unter 8000 Stapesoperationen wurde sie von House & Patterson (1964) nur 2mal gefunden. Nach Hough (1958) ist sie jedoch mit dem kleinen zarten Gefäß identisch, das man bei Operationen sehr häufig über die Mitte der Steigbügelfußplatte ziehen sieht. Aus der A. carotis interna kommend, perforiert es den Boden der Paukenhöhle und zieht über das Promontorium und zwischen den Stapesschenkeln hindurch in den Facialiskanal hinein. Größere persistierende Gefäße erreichen mit diesem Nerven zusammen das Endocranium wo sie sich in Äste für die Versorgung der Dura aufzweigen.

Wir selbst haben bei der Operation von etwa 1000 Schalleitungsschwerhörigkeiten bei reizlosem Mittelohr die zumeist otosklerosebedingt waren, nur 1mal eine Stapesmißbildung bei persistierender A. stapedia gesehen.

Bei dem 31 Jahre alten Patienten B. E., Krbl.-Nr 244 /64, wurde wegen einer seit der Jugend bestehenden Schalleitungsschwerhörigkeit rechts eine Probetympanotomie vorgenommen. Ohrentzündungen waren in der Anamnese nicht angegeben worden. In der reizlosen Pauke fand sich als Stapesrudiment lediglich ein gekrümmter kleiner Knochenspan, der auf dem hinteren Rand des ovalen Fensters knöchern fixiert war und dessen oberer Teil zu einem deformierten Stapes-Amboßgelenk zog. Die Stapediussehne war erhalten. Über die normal angelegte und bewegliche Fußplatte zog von caudal nach cranial genau in der Mitte ein größeres Gefäß, bei dem es sich mit großer Wahrscheinlichkeit um die persistierende A. stapedia handelte. Das Gefäß wurde elektrokoaguliert und durchtrennt, die Fußplatte entfernt. Der Defekt in der Schalleitungskette wurde durch eine Fett-Bindegewebe-Prothese nach Schuknecht überbrückt, nachdem zuvor das Stapesrudiment hinter dem ovalen Fenster entfernt worden war. Das funktionelle Ergebnis des Eingriffes zeigt das Audiogramm 2 a.

Interessant erscheint uns im vorliegenden Fall neben der Persistenz der A. stapedia die Existenz eines ovalen Fensters mit beweglicher Fußplatte neben einem nicht mit dieser in Verbindung stehenden Steigbügelrudiment. Es stellt sich hier die Frage, wodurch die Ausbildung des ovalen Fensters in der embryonalen Labyrinthkapsel induziert worden sein mag. Die extrahierte normal dicke Fußplatte, deren histologischer Untersuchungsbefund leider nicht vorliegt, bestand offensichtlich aus der branchiogenen knöchernen und der labyrinthogenen knorpeligen Schicht. Ursprünglich muß also eine embryonale Verbindung des Stapesrudiments mit der beweglichen Fußplatte vorhanden gewesen sein. Die Abspaltung eines Stapesteil von der Fußplatte findet im vorliegenden Fal ihre Erklärung wahrscheinlich in der Persistenz der starken A. stapedia. Die Zunahme des Gefäßkalibers mit dem Wachstum des Versorgungsgebietes führte wahrscheinlich bereits im frühen Embryonalstadium zur Drucknekrose der Stapesschenkel In ähnlichen Fällen vor geteiltem Steigbügel ohne persistierende Arter e jedoch mit ausgebildetem Foramen ovale (Gi. 1959), mag das die Mißbildung verursachend einst starke Gefäß später obliteriert sein.

Nach Beickert (1961) sind totale Stapesaplasien stets mit einer Aplasie des ovalen Fensters kombiniert. Bei solchen Fensteraplasien werden zuweilen auch Reste dysplastischer Steigbügelschenkel gefunden (Ombrédanne, 1959 Henner 1960 Hough 1958 1963) Diese Kombination von Fehlbildungen findet ihre Erklärung im Mechanismus der normalen Embryogenese des Steigbügels. Wenn das Blastem völlig fehlt oder sich durch die interponierte A. stapedia nicht physiologisch entwickeln kann, fehlt seine induktive Wirkung auf die Ohrkapsel, und es kommt nicht zur Herausbildung der Lamina stapedialis. Es resultiert der Verschluß des ovalen Fensters durch eine dicke Knochenplatte, die keinerlei Verbindung mit Stapesrudimenten hat.

Isolierte Fehlbildungen des Stapes zu columellaähnlichen Gebilden wurden von verschiedenen Autoren beobachtet. Tomka beschrieb bereits 1895 zwei selbst beobachtete Fälle und referierte in seiner Arbeit eine Fülle von dies-

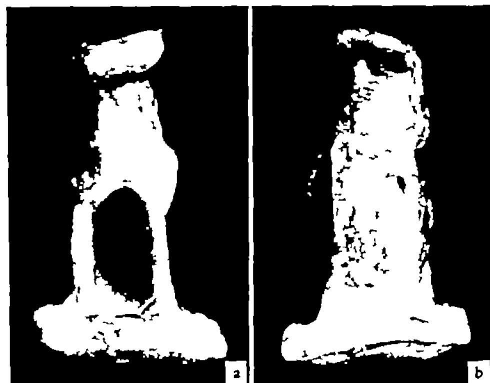


Abb 1 (a, b) Columellaförmiger Steigbügel. Das plumpe Knöchelchen ist auf einer Seite rillenförmig ausgehöhlt und die Fußplatte kleiner als normal.

bezüglichen Veröffentlichungen, zumeist aus dem anatomischen Schrifttum. Weitere Mittei-
-en dieser Art kamen von Alexander & Bě
(1921), Hörbst & Sauser (1937), Plester
961), Edwards (1964) und Banham (1966)

Auch wir konnten eine solche Beobachtung machen. Bei der 55jährigen Patientin E. W. Krbl. N 1907 68 wurde eine Probetympanotomie rechts wegen einer unklaren Schalleitungsschwerhörigkeit vorgenommen. Dabei zeigte sich, daß der mißgebildete Steigbügel praktisch nur aus einem auffällig starken und relativ steilgestellten hinteren Schenkel bestand und zu einer Fußplatte führte, die etwa die Größe einer hinteren Fußplattenhälfte hatte. Der vordere Teil dieser kleinen Fußplatte zeigte kein typisches Ringband, die Beweglichkeit war deshalb hochgradig eingeschränkt. Auffällig war auch das plumpe Stapesköpfchen (Abb. 1 *a, b*). Im Bereich der vorderen Hälfte des ovalen Fensters war keine Fußplatte angelegt.

Diese Form der Stapesmißbildung ist wohl so zu deuten, daß das embryonale Blastem die A. stapedia nicht gabelförmig sondern nur auf einer Seite umwachsen hat und dadurch ungeteilt blieb. Hat ein Schenkel während der Embryonalentwicklung die Fußplatte nicht erreicht, so steht der andere gut ausgebildete in der Regel auf einer rudimentären kleineren Platte (Altmann, 1965). Sie entsteht durch die nur kleine Verschmelzungsfläche zwischen Ohrkapsel und branchiogenem Stapesblastem. Ombrédanne (1959) sah u. a. einen columellaförmigen Stapes, bei dem die typische Rillenbildung fehlte. Das Fehlen dieser Schenkelrinnen kann auch beim sonst normal entwickelten Steigbügel vorkommen.

Therapeutisch haben wir in diesem Falle das Stapesrudiment entfernt und eine Schuknecht-Prothese mit sehr kleinem Bindegewebsläppchen zur Interposition verwendet. Das funktionelle Ergebnis zeigt das Audiogramm 2 b.

Daß außer den bisher genannten Ursachen auch der N. facialis die normale Entwicklung des Steigbügels negativ beeinflussen kann, soll mit dem nachfolgend geschilderten Fall belegt werden.

Bei der 23jährigen Patientin B. P. Krbl. N. 1541/64, handelte es sich um eine Dysostosis mandibulofacialis (Franceschetti). Die Abb. 3 zeigt die typischen Fehldifferenzierungen der mesenchymalen Derivate des 1. und 2. Kiemenbogens wie Mikrognathie mit breiter Mundspalte und antimongoloide Lidachsenstellung mit rechtwinkliger Abnickung des Unterlides. Die häufig gleichzeitig vorkommende Aplasie der Ohrmuschel fehlt. Die Patientin litt seit Geburt an einer hochgradigen Schalleitungsschwerhörigkeit, die die Annahme von Mittelohrmißbildungen nahelegte. Die Spiegeluntersuchung zeigte einen außerordentlich engen Gehörgang und ein Trommelfell, in dem die Kontur des Hammergriffes und des kurzen Hammerfortsatzes fehlte. Die stark vergrößerte Pars flaccida nahm fast ein Drittel der gesamten Trommelfellfläche ein. Da die Patientin auf dem linken Ohr zum Zweck der Hörverbesserung in einer anderen Klinik bereits vor Jahren radikaloperiert worden war, ließ sich ein genauer Befund dieser Seite nicht mehr erheben. Eine Hörverbesserung war seinerzeit nicht erreicht worden. Bei der von uns am rechten Ohr vorgenommenen Probetympanotomie fanden wir als Rudiment von Hammer und Amboß nur ein rundliches, bewegliches Knochengebilde im Kuppelraum oberhalb des horizontalen Bogenganges. Hammergriff und langer Amboßschenkel fehlten. Der N. facialis zog frei durch die enge Paukenhöhle. Sein vorverlagertes Knie bedeckte den hinteren Teil des Foramen ovale, das in diesem Teil zum Vestibulum hin eine dicke Knochenplatte aufwies. Eine dünne gebogene kleine Knochenlamelle lag dem Nerven von caudal her an. Sie wirkte zuerst wie ein Fragment der normalerweise vorhandenen knöchernen Nervenbedeckung. Die genaue Kontrolle zeigte jedoch, daß sie in einer winzigen beweglichen Fußplatte endete, die das restliche kleine Foramen ovale zum Vestibulum hin abschloß.

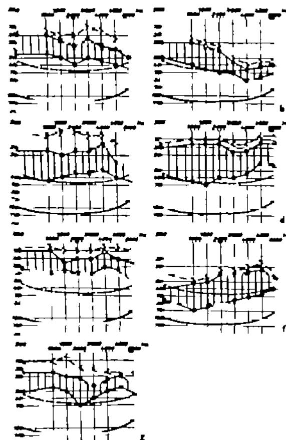


Abb. 2 Das funktionelle Ergebnis der hörverbessernden Operationen.



Abb. 3 23jährige Patientin mit hochgradiger angeborener Schalleitungsschwerhörigkeit bds. bei Franceschetti-Syndrom.

Es handelte sich hier offensichtlich um einen völlig dysplastischen Steigbügel, der weder mit dem deformierten Amboß noch mit dem Trommelfell Kontakt hatte (Abb. 4 *a*, *b*). Die Annahme liegt nahe, daß bei der Genese dieser Fehlbildung der atypisch verlaufende N. facialis eine causale Rolle spielte. Durch die ungewöhnlich ventrale Lage des Nerven in der Paukenhöhle wurde die regelrechte Verschmelzung des

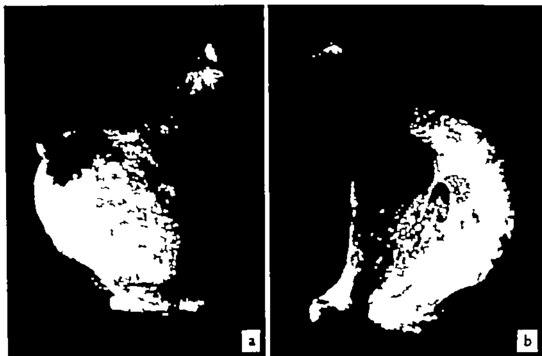


Abb 4 (*a, b*) Dysplastischer rechter Steigbügel der Patientin on Abb. 3 Die konkave Flache der leicht gebogenen Knochenlamelle (Abb. 4 *b*) lag von ventral dem N facialis an, der frei durch die Paukenhöhle zog und den großten Teil der ovalen Fensternische bedeckte. Die sehr kleine Fußplatte ist deutlich zu erkennen, ein Köpfchen ist nicht ausgebildet.

branchiogenen Stapesblastems mit der Ohrkapsel verhindert. Die Steigbügelanlage wurde ahrscheinlich durch den immer dicker wer Nerven so weit platt gedrückt, daß nur ' *schmaler Ausläufer der dünnen Steigbügel*lamelle in der Ohrkapsel die Differenzierung einer winzigen Lamina stapedialis induzieren konnte

Tabor (1961) beschrieb einen ähnlichen Fall von vorverlagertem N facialis, der ohne knöcherne Bedeckung frei durch die Paukenhöhle zog. Bei seiner Patientin, einem 14jährigen Mädchen, hatte der Steigbügel rudimentäre Schenkel, die Fußplatte fehlte völlig.

Therapeutisch haben wir in diesem Fall nach Entfernung des Stapesrudiments eine entsprechend geformte Knorpelcolumella zwischen Trommelfell und Fensterrest interponiert. Wir konnten dadurch eine teilweise Reduzierung der Schalleitungskomponente erzielen. Nach unseren jetzigen Erfahrungen wäre die Verwendung einer kombinierten Knorpel-Draht-Bindegewebe-Columella (Gerhardt, 1969) in diesem Fall wahrscheinlich günstiger gewesen. Das von uns erzielte Ergebnis zeigt das Audiogramm 2 c.

Neben den bisher geschilderten Stapesdysplasien findet man solche, die sich nicht auf die bereits genannten Entstehungsmechanismen zurückführen lassen. Hier handelt es sich offensichtlich weder um eine lokale Irritation der Stapesanlage durch Gefäße oder Nerven noch um eine endogene Fehldifferenzierung des Blastems. Man beobachtet vielmehr eine Überschußbildung in Form von höcker und spießförmigen Exostosen an Fußplatte oder Schenkeln und bei anderen Steigbügeln einen partiellen oder totalen lamellären knöchernen Verschluß des normal weiten Intercruralraumes (Abb 5)

Diese morphologischen Varianten beeinflussen das Hörvermögen nicht. Sie entstehen durch überschießende Ossifikation des den knorpligen Steigbügel umgebenden embryonalen Mesenchyms. Solche abnormen knöchernen Spangen werden mitunter auch als Residuen entzündlicher Prozesse gefunden, ferner vielfach beim endemischen Kretinismus (Altmann, 1933). Er

reichen diese knöchernen Spangen die Paukenhöhlenwand, so kommt es zur Fixierung des betreffenden Ossikulums, deren einziges Symptom die angeborene Schalleitungsschwerhörigkeit ist.

Wir beobachteten eine solche Fehlbildung bei einer 13jährigen Patientin (S. F. Krbl. Nr. 1982/66). Es ist bemerkenswert, daß in der Familienanamnese dieser Patientin eine nicht entzündlich bedingte Schwerhörigkeit mehrfach angegeben wurde. Sowohl der Großvater mütterlicherseits als auch die Mutter und deren Schwester litten seit der Kindheit daran. Bei unserer Patientin bestand eine Schalleitungsschwerhörigkeit beiderseits von etwa 40 dB im ganzen Frequenzbereich. Für eine entzündliche Genese fand sich weder anamnestisch noch bei der klinischen Untersuchung ein Anhalt. Die zunächst vorgenommene Probetympanotomie ergab, daß an einem sehr dünnen und deformierten langen Amboßschenkel ein Stapes inserierte, bei dem man zunächst drei Schenkel zu erkennen glaubte. Der vorderste „Schenkel" stellte sich als eine Knochenbrücke heraus, die vom Crus rectilineum zum Promontorium zog. Der eigentliche vordere Schenkel war in Richtung Knochenbrücke verzogen und inserierte atypisch schräg auf einer relativ kleinen Fußplatte. Nach Frakturierung der Knochenbrücke zeigte es sich, daß die Fußplatte frei beweglich im ovalen Fenster angelegt war.



Abb. 5. Der Intercruralraum ist teil eise durch eine Knochenlamelle verschlossen. An der Fußplatte otosklerotische Herde

Auf den Abbildungen *6 a* und *6 b* sind das zur Hälfte knöchern verschlossene Spatium intercrurale sowie der Ansatz der abgebrochenen Knochenbrücke zu erkennen. Die Familienanamnese läßt daran denken, daß bei der Patientin eine erbliche, also genbedingte Fehldifferenzierung des Stapesblastems vorliegt.

Eine weitere Gruppe von Steigbügelanomalien bilden die connatalen stapedo-vestibulären Ankylosen, die Agazzi (1965) zu den „großen Dysplasien" unter den verschiedenen Formen der „Atresia auris congenita" zählt. Sie sind durch eine fehlende Differenzierung des Ringbandes aus der vorknorpeligen Labyrinthkapsel charakterisiert. Es ist noch unklar, weshalb es hier trotz erfolgter Verschmelzung von branchiogenem Stapesblastem und Ohrkapsel zu solchen *Hemmungsmißbildungen* kommt. Tabor (1961) meint, daß zur späteren Bildung von ankylosierenden Knochenbrücken das Zurückbleiben nur weniger präcartilaginärer Restzellen im Ringbandbereich genügt. So läßt sich auch erklären, daß es sehr verschiedene Grade der Fußplattenfixierung gibt. Die Skala reicht von geringen perlhanlären Fixationen bis zum Ersatz des ganzen Ringbandes durch dicken Knochen. Über solche Beobachtungen wurde in den letzten Jahren von Holmgren (1958), de Witt (1958), Hough (1958), Ombrédanne (1959), Lindsay *et al.* (1960), Plester (1961) und Wolff (1964) berichtet. Das funktionelle Korrelat war bei allen diesen Fällen eine angeborene Schalleitungsschwerhörigkeit von 50 bis 60 dB im gesamten Frequenzbereich.

Wir konnten entsprechende Beobachtungen bei 2 von unseren Patienten machen. Bei der 19jährigen Patientin W. K., Krbl.-Nr. 1185/63 inserierten auffällig plumpe Crura auf einer vom umgebenden Labyrinthknochen nicht abgrenzbaren festfixierten Knochenplatte. Durch Frakturieren der Schenkel, Bildung einer Öffnung zum Vestibulum, die mit einem Bindegewebsläppchen verschlossen wurde, und Reposition der Schenkel auf dieses Bindegewebsläppchen konnten wir praktisch die ganze Schalleitungsstörung beseitigen. Das prä- und postoperative Audiogramm bei dieser Patientin zeigt die Abb. 2 d.

Ein weiterer 13jähriger Patient (K. J. M., Krbl. N 1718/66) bot praktisch den gleichen Befund. Außerdem fehlte hier jedoch das typische Stapes-Amboßgelenk. Beide Ossikula waren lediglich durch





Abb 6. (a, b) Der Rest der vom vorderen Schenkel des Steigbügels zum Promontorium ziehenden Knochenbrücke ist deutlich zu erkennen, der Intercruralraum teilweise verschlossen.

lockeren fibrösen Strang miteinander verbunden. Nach Entfernung des Stapesbogens, Bildung eines neuen Fensters zum Vestibulum und Überbrückung des Kettendefektes mit einer Draht Bindegewebe-Prothese ...h Schuknecht konnten wir auch in diesem Fall ... gutes funktionelles Ergebnis erzielen. Audiogramm ...

Wenn sich auch aus der genauen Kenntnis der normalen Entwicklung der Stapesanlage der Entstehungsmechanismus von bestimmten Fehlbildungen mit großer Wahrscheinlichkeit herleiten läßt, so gibt es doch immer wieder Fälle, in denen dies schwierig ist.

Wir möchten als Beispiel den Befund bei unserem Patienten S. W Krbl. Nr 977 68, anführen. Bei dem Patienten bestand seit Geburt eine hochgradige kombinierte Schwerhörigkeit beiderseits Gehörgang und Trommelfell waren normal angelegt. Auffällig war nur die starke Neigung des Trommelfells gegen den Gehörgangsboden, wie man es sonst nur bei Säuglingen zu finden pflegt. Nach Eröffnung der Pauke zeigte es sich, daß der Steigbügel mit seinem hinteren Schenkel auf der normal *dicken Labyrinthwand stand.* Es fehlte selbst die Andeutung einer Nische zum ovalen Fenster Der vordere Schenkel war nur in seinem oberen Drittel knöchern angelegt. Von hier aus zog ein bindegewebiger Strang, etwa von der Dicke eines normalen Steigbügelschenkels, zu einem winzigen Fenster (Durchmesser etwa 1/2 mm) im Vestibulum dicht unterhalb des N facialis. Wir entfernten das Stapesrudiment und erweiterten die Öffnung zum Vestibulum so weit, daß ein T flon-Piston zwischen den normal geformten langen Amboßschenkel und das neu gebildete Fenster interponiert werden konnte Das erzielte funktionelle Ergebnis zeigt das Audiogramm 2 f.

Die Atrophie und ausbleibende Verknöcherung am vorderen *Stapesschenkel* könnte wiederum die Folge einer asymmetrisch gelegenen *A. stapedia sein,* die auch die *Induktion einer* entsprechend großen Fußplatte in diesem Bereich verhindert hat. Unklar bleibt jedoch, warum im Bereich des hinteren weit besser ausgebildeten Steigbügelschenkels die Induktion einer Fußplatte völlig unterblieb

Als Beispiel für das Vorkommen von Kombinationen der beschriebenen Fehlbildungen möchten wir den Befund bei einer weiteren Patientin aus unserer Klinik schildern.

Die 13jährige Patientin L. G Krbl.-Nr 468/65 wurde uns wegen einer mittelgradigen vorwiegend schalleitungsbedingten Hörstörung überwiesen. Die klinische und röntgenologische Untersuchung ergab

keinen Anhalt für die Ursache der Hörstörung. Bei der Probetympanotomie zeigte sich, daß dicht ober halb der Stapediussehne ein pyramidenförmiger Knochenvorsprung von der hinteren Paukenwand zum Stapeshals zog und diesen fixierte. Eine zweite Knochenspitze zog vom Facialiskanal her ebenfalls zum Bereich des Stapesköpfches und verstärkte die Fixierung noch. Der Stapes selbst hatte nur einen hinteren, relativ steil gestellten Schenkel, der annähernd zentral auf einem relativ kleinen (1 / 1 / mm) angedeuteten Fußplattenbereich inserierte. Diese Fußplatte hatte jedoch kein Ringband und war im ganzen Umfang knöchern mit der umgebenden Labyrinthwand ver bunden. Wi entfernten beide Knochenspitzen und das Stapesfragment. Im Bereich der angedeuteten Fußplatte wurde ein Fenster zum Vestibulum angelegt und der Defekt der Schalleitungskette durch eine Draht-Bindegewebe-Prothese überbrückt. Das funk tionelle Ergebnis zeigt das Audiogramm g.

In diesem Fall bestanden also neben den bereits erwähnten knöchernen Überschußbildungen peristapedial eine Aplasie des vorderen Steigbügelschenkels und eine connatale stapedovestibuläre Ankylose der wohl primär angelegten kleinen Fußplatte unter dem hinteren Schenkel.

Abschließend möchten wir feststellen, daß wir die beschriebenen 8 Stapesmißbildungen bei der Operation von etwa 1000 Schalleitungsschwerhörigkeiten bei reizlosem Mittelohr zumeist Otosklerosen, gefunden haben. Die Häufigkeit liegt also etwas unter 1%. Im gleichen Krankengut fanden wir 5mal eine Aplasie des M. stapedius (0,5%).

SUMMARY

After short description f the stapes development the author reports 8 cases with stapes malformation found in about 1000 patients suffering from disturbance of sound conduction of primary unknown origin, mainly otosclerosis. The possible genesis of these malformations is then discussed. Aplasia of the stapedius muscle was found in 0.5% (5 ears) of the same number of patients. From the functional results achieved by operation it can be concluded that the common methods presently used in stapes surgery are quite successful in most cases.

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RECONSTRUCTING CONDUCTION IN THE EAR WITH NO OSSICLES

Middle Fossa Epidural "L" Strut
Preliminary Report

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Abstract A new method is described for restoring conduction in middle ear containing no ossicles. Use is made of an autogenous "L bone strut or Teflon prosthesis as an "incus" replacement. The latter is stabilized in tegmen tympani fenestra to avoid slippage, and it also aids in the formation of middle ear space. Around the "incus wire prosthesis is crimped and suspended to make contact with the oval window Two cases are 2 / years after operation and one case 18 months postoperatively

There are currently two diametrically opposed schools of thought regarding the surgical management of cholesteatoma affecting the entire tympanic cleft. Our teachers Shambaugh, Jr (1967), Baron (1967) and many others are advocates of "taking down" the posterior canal wall and exteriorizing the mastoid cavity in order to avoid the "trapping of cholesteatoma and insuring direct visual surveillance of the cavity Sheehy & Patterson (1967) and his associates, in those cases where there is not an absolute indication to take down the posterior canal wall, advocate intact canal wall surgery with the aid of the "facial recess window" for visualization of the middle ear This modality demands meticulously exacting dissection, technical experience and relentless patient follow-up Their early follow up is most encouraging However for the resident in his early training period posterior canal wall preservation would not be the treatment of choice

When we are faced with a situation in which no ossicles are present either due to the disease process, their removal, or a radical mastoid cavity of long standing, restoration of conduction presents a significant problem. Proctor (1964) in his excellent critical review of 122 cases of Type IV Tympanoplasty reported that 53% of his patients had a post-operative air bone gap of 30 dB or less. Waltner (1966) reports only 30% serviceable hearing results in his Type 4 tympanoplasties. Sheehy and House (Sheehy 1965 House & Sheehy 1963) also allude to their poor results via same. Siedentop & Brown (1966) report a high incidence of polyethylene columella struts (drum to foot plate) extrusion and long-term deterioration of initially successful hearing results Bauer (1966) augments cortical bone to re-establish ossicular continuity with that of tympanic membrane in the absence of varying degrees of ossicular components. Waltner reports encouraging results with his "Dumbbell Tympanoplasty" and reports no extrusion of the prosthesis in four patients at the end of [illegible] He claims that the changes of slippage [illegible] mal. Waltner (1966) further [illegible]

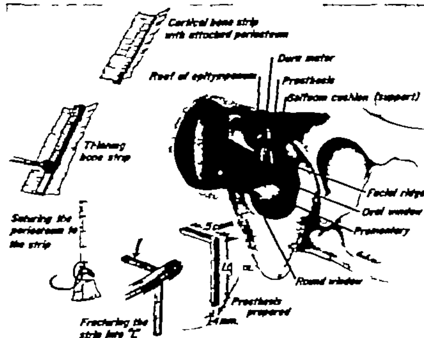


Fig 1 Diagram illustrating autogenous bony epidural "L" strut prosthesis.

technique by using a 5 mm long narrow bar of cartilage in the upper loop of the stainless steel wire and reports favorable results. Sheehy and his collaborators are reluctant to remove all ossicular structures that may be marginally affected by the disease process. They utilize both auto- and homograft ossicles which have been eansed and autoclaved for restoring conducon the middle ear as well as tragal cartige as a *columellar strut. Their incidence is* remarkably low

It would appear that a uniformly accepted method for the restoration of transtympanic conduction in the ear with no ossicles is lacking. Essentially the problem is one of obtaining an intact mobile drum which will transmit the sound impulse to the footplate Slippage of the interposed "ossicle or strut" and extrusion of same has accounted chiefly for the inconsistent results which have been reported.

In an attempt to overcome this, the authors originally planned to insert a cortical piece of bone in a hammock fashion in a fascial graft of the tympanic membrane and in a staged procedure to suspend a prosthesis from this to the footplate. Further to work on cadavers, the following modality was developed.

TECHNIQUE

Sufficient overhanging bone is drilled away in the postero-superior portion of external canal and middle ear to expose the epitympanum and tegmen tympani. An approximately 2 × 3 mm fenestra is made in the tegmen just above the level of the horizontal facial ridge and *roughly in the same antero-posterior plane* as the oval window The exposed dura is bluntly elevated using a stapes elevator on narrow vaginal gauze. A piece of mastoid cortical bone is obtained and fashioned to an approximately 1.5 × 0.2 × 0.2 cm strut. The periosteum is left attached on its cortical aspect and the overlapping periosteum is enveloped around its undersurface and sewn in the longitudinal midline axis. At its mid-junction the bone strip is fractured. The attached periosteum serves to make this a "greenstick" fracture The one limb is inserted into the fenestra and slight shortening by 12 mm may be necessary at each end. Gelfoam is gingerly packed into the fenestra. The strut now projects into the middle ear hanging as a lever A piece of bone may be placed under the strut, adjacent

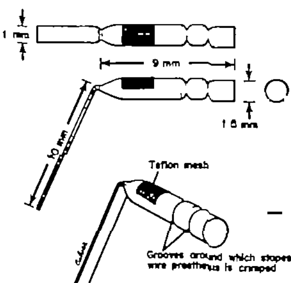


Fig 2 Diagram f Teflon prosthesis.

to the fenestra for support. Over this a fascial graft is placed.

CASE REPORTS

1 E. B a 38-year-old Negro male with fourteen-year history of a running" left ear when seen in April, 1967 was found to have a moist left ear with an attic cholesteatoma. In addition to a hearing loss (Fig. 3), the patient complained of tinnitus and being off balance. On April 17 1967 a left radical mastoidectomy was performed via a post-auricular incision. An autogenous bone "L" strut was placed as described across the epitympanum and a fascial graft placed upon this new incus" The distal end of the strut was not indented for the lodgement of the stapes prosthesis as was done in subsequent cases using the prefabricated Teflon L struts (Fig. 2) On June 21 1967 inspection revealed a dry ear and a "take" of the graft. Under local anesthesia a tympanotomy was done which showed the transepitympanic distal end of the strut to be overlying the oval window and very nicely movable to the gentlest touch. A prefabricated Teflon piston was suspended to the oval window from the bone strut but due to width of the strut, the prosthesis could not be crimped The patient s hearing postoperatively improved to the point when it became clinically serviceable and maintained same However on January 15 1968 there was evidence of squamous epithelium retraction in the aditus area and canal stenosis. Under local anesthesia recurrent cholesteatoma was removed from the aditus *and lateral epitympanum. The bone strut had* maintained its position and mobility with a thickened fibrous band connecting it to the footplate The wire portion of the Teflon prosthesis had been displaced from the bone strut and was therefore removed while the canal skin was excised posteriorly Since then the hearing had been maintained, the ear had been dry and the external canal had contained normal cerumen However marked canal stenosis recurred as well as keloid over the postauricular incision. The ear was reoperated upon in May 1969 At this time it was seen that the strut had maintained its position, viability and mobility The keloid in the canal, mastoid bowl, and postauricularly was widely excised along with 1 cm of conchal cartilage and a skin graft applied. 600 R was given to the area the day following operation and at 6 months post operatively the keloid has not reformed. The meatus is widely patent and the bowl is well epithelialized. The drum is adherent to the L" strut which is freely movable The air-bone gap is slightly greater than the reading record-

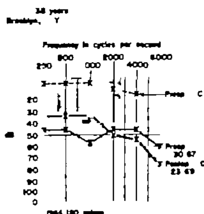


Fig 3 Pre- and post-operative

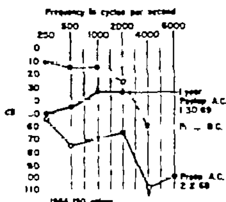


Fig 4 Pre and post-operative audiogram of case 3.

ed at 1.23 69 but with the absence of stenosis the stapes replacement prosthesis can be repositioned at a future date at the patient s convenience.

2. G H a 47 year-old veteran white male first presented with a long-standing dry right radical mastoid cavity and a chronic "running" left ear Audiograms revealed severe bilateral con ductive hearing loss. On June 30, 1967 the mastoid bowl epithelium was excised in toto The entire medial wall of the middle ear was " ...d of its epithelium The eustachian ori- ... showed healthy mucosa. A cortical bone ... with its periosteum was fashioned and utilized as outlined. Silastic sponge was placed in the hypotympanum and a fascial graft positioned over the strut and silastic. Healing was uneventful and examination on 12.29 68 revealed a ?0 anterior-inferior perforation. Through the latter the middle ear cavity could be seen to be lined by healthy looking mucosa. The projecting portion of the bone strut could be seen to be well adherent to the mobile drum as is the malleus handle in a normal tympanic membrane except that it lay directly over the "footplate area" Audiograms indicated the air and bone conduction levels to correspond to the preoperative levels, and further surgery is planned.

3 B K. was a 60-year-old white male with bilateral "running ears" and marked conductive hearing loss since the age of 5 years. Examination of both ears revealed completely deficient tympanic membranes and middle ears filled with granulations, cholesteatoma and a thick muco-purulent discharge The hearing in the right ear demonstrated in audiogram (Fig. 4) On March 17 1967 surgery was attempted under general anesthesia. The procedure was discontinued due to poor capillary filling of the extremities and the absence of central venous monitoring. On February 5 1968 under local anesthesia a radical mastoidectomy was completed for extensive cholesteatoma involving the tympanic cleft. No stapes super structure was present and there was a 40% defect in the tegmen tympani. A preformed Teflon "L" strut was inserted into the tegmen fenestra and from the distal end a 5 mm number 36 wire prosthesis was suspended to the footplate and crimped around the Teflon strut. The middle ear was packed with gelfoam and a round piece of canal skin was placed over the Teflon epidural strut extending just beyond the annulus tympanicus. The sclerotic mastoid bowl was not grafted as it was planned for this cavity to epithelialize separately from the drum. The cavity and the external canal were packed with gelfoam. Healing was uneventful and the hearing continued to improve as in audiogram (Fig. 4) The ear has remained dry following slight discharge for a brief postoperative period The tympanic membrane at first was thick until gradually 9 months postoperatively it became thinner and, in part, transparent. The wire prosthesis could be identified and seen to be nicely crimped around the "Teflon incus" the outline of which was both palpable and distinguishable, but covered. With swallowing and Valsalva the middle ear cavity was seen to inflate with air while the drum was mobile The patient s hearing was remarkably socially adequate (Fig. 4) At 15 months after operation the patient experienced an acute episode of rhino-sinusitis and right otitis media which resolved following conservative management with antibiotics and decongestants. At 17 months the terminal bulbous

portion of the Teflon prosthesis protruded through the drum membrane However behind this portion the membrane is adherent to the prosthesis, the drum moves very well and the bearing at 18 months has maintained the acuity of 1.30 69 Since the patient currently has car diovascular disease, it is planned to shorten the Teflon prosthesis by nipping off the terminal portion at the second groove and reinforcing the under surface of the drum with fascia when the patient s condition allows. The patient s left ear was identical in pathology and hearing def icit to the right ear Under local anesthesia an identical procedure was performed on 2.5 69 except that the Teflon prosthesis used was shorter At 10 months the healing process is the same as occurred in the right ear Gentle postoperative inflation of the middle ear by the Valsalva maneuver was encouraged in this case The patient did complain of "throbbing" in his left ear at night which at 6 months is minimal.

COMMENTS

Fashioning the cortical bone strut is a laborious procedure. Consequently a prefabricated Teflon "L strut was utilized in case 3 and in subsequent cases. However we are in the process of having made an instrument that will readily construct from a bone segment the de sired bone strut similar in outline to the Teflon "incus A series of both the cortical bone and Teflon-Supramid struts for comparative evaluation is in progress and a report will be submitted in due course

Our limited experience in four ears seems to have been uniform, as was the case in subsequent ears which are not included, due to only a few months follow-up. In cases 1 and 3 as in the cases that followed, it was necessary to control the process of healing in the bowl and meatus by repeated $AgNO$ cautery and packing. This took place over an approximate period of 2 months at which time all ears became and remained dry The hearing began to become significantly improved at 3–4 months In no case was there any evidence of meningeal infection presumably due to the tolerant nature of the dura mater In all case the new drum became adherent to the L strut which maintained its mobility and did not become fixed to the facial ridge A middle ear space which clearly demonstrated its infla tion with air via the eustachian tube was achieved in all cases. It appears that there is no need to stage this procedure and gentle post operative valsalva would seem beneficial.

The throbbing which occurred in case 3 and which has persisted minimally at night in the left ear is perhaps due to transmission from the vessels in the middle fossa. Using a short r Teflon strut with only one of the grooves (Fig. 2) will possibly avert the complication in the right ear of case 3 At the suggestion of Dr Jürgen Tonndorf we are slightly modifying the Teflon prosthesis.

ACKNOWLEDGMENTS

Acknowledgment is made to Dr R. Bellu n for taking pre- and postoperative movie stri s of case 3 and postoperative strip of case 2 to Dr B Kapila and Dr M Patel, my former residents, as cases 1 and 2 respectively were their ward patients to Mr J Halligan and staff of Becton and Dickinson, New Jersey for constructing the Teflon prosthesis and to Edward Week and Co who are in the process of mak ing an instrument to construct the cortical bone strut.

The illustrations were done by Bruce Culver of the Dept. of Medical Illustrations and Photography State University of New York, Downstate Medical College, Brooklyn, New York.

ZUSAMMENFASSUNG

Eine neue Methode zur Wiederherstellung der Leitung in einem Mittelohr ohne Gehörknöchelchen wurde beschrieben. Sie benutzt eine körpereigene, "L -förmige Knochenstütze oder eine Teflon-prothese als Ambossersatz. Um ein Verrutschen zu vermeiden, wird die Letztere in einem Fenster im Dach der Paukenhöhle befestigt, dies hilft gleichzeitig bei Bildung eines Mittelohrraums. Um den

eine Drahtprothese gepreßt und so aufgehängt, daß eine Verbindung mit dem ovalen Vorhofsfenster besteht. Die Operation von zwei Patienten liegt jetzt 2 / Jahre und die von einem dritten 18 Monate zurück.

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THE IDENTIFICATION AND TOPOGRAPHICAL LOCALISATION OF THE OLFACTORY EPITHELIUM IN MAN AND OTHER MAMMALS

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Abstract Examination in ethanol of fixed specimens of nasal mucosa in incident light reveals the precise localisation of the olfactory epithelium in man and other mammals. It is demonstrated that the olfactory margin in man is regular at about 2 years the margin begins to acquire an irregularity as degeneration of the olfactory organ (epithelium) sets in.

In recent years a number of papers have dealt with the ultrastructure of the olfactory region (de Lorenzo, 1957 1960 1963 Frisch, 1964 1965 1967 Graziadei, 1964 Reese, 1965 Okano, 1965 Andres, 1966 Seifert & Ule, 1967 Okano et al., 1967) Research is mainly directed towards ultrastructure relatively less attention is focused on this region as a whole and on the spatial arrangement of its cellular components, which is of importance as the basis of function.

The *conventional method of identification* of the olfactory epithelium and determination of its extent is that of decalcification, embedding and serial sectioning of nasal tissue a reconstruction of the sections and subsequent plotting in schematic drawings complete the procedure (von Brunn, 1892 Kolmer 1927 Le Gros Clark, 1946 1951 Muller 1955 Negus, 1958) Other investigators (Read, 1908 Smith, 1941) used other indirect means to assess the extent of the olfactory area in man. These methods are time consuming and prone to inaccuracies.

Macroscopic methods and those of use in conventional histology in studies of the nasal mucosa have been relatively neglected Methods of investigation for use not only in experimental studies but also in the evaluation of histopathological changes that occur in the olfactory region of man are required.

A simple and direct method of visualisation *in toto* of the olfactory organ (epithelium) in animals and in man is presented in this pap

METHOD

After decapitation, the ethmoturbinal complex of bone (Fig. 1) or nasal septum of an animal is excised and immersed in a chilled solution of 1.5% osmic acid buffered with Veronal acetate at pH 7.2. The specimen is allowed to fix for 2 hours. It is then rinsed with and placed in 70% ethanol. An hour later the submerged specimen can be examined in a Petri dish under an operating or stereomicroscope by incident light.

The nasal mucosa of man *post mortem* may be similarly treated

OBSERVATIONS

A striking colour contrast appears which corresponds to the two well known histological parts of the nasal mucosa. In the guinea and man the *olfactory epithelium* appear

This paper is part of thesis presented at Trinity College, Dublin. in September 1968



Fig 1 A freshly prepared dissection towards the left side from the sagittal plane of the skull of guinea pig. The ethmo-turbinal complex *ET* in the posterior recessed part of the nasal cavity shows the medial aspect of endoturbinals 1 –, 3 4 the nasoturbinal *NT* and maxillary turbinal *MT* lie anteriorly within the respiratory chamber of the nasal cavity X marks the region of the posterior choanae. Arrow in subethmoidal part of the nasopharyngeal duct. *AN* anterior nares; *N* nasal bone· *CP* cribriform plate· *LT* lamina terminalis (subethmoidal shelf of bone); *OB* olfactory bulb· *C* cerebrum *I* incisor tooth, *Mo* molar tooth socket.

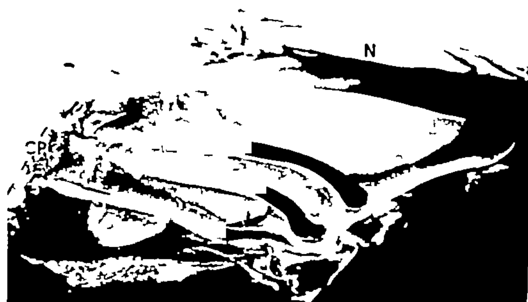


Fig 2 Ethmo-turbinal complex of bone guinea pig, as seen in ethanol in incident light after immersion in 1.5% osmic acid. The endoturbinal are shown to bear olfactory epithelium (light grey) but are not entirely covered by it, their anterior ends (black) are invested in ciliated respiratory epithelium. Note the sharp rather regular contour of the 'olfactory margin on all turbinals. *N* nasal bone· *CP* cribriform plate· *F* fossa for the olfactory bulb.

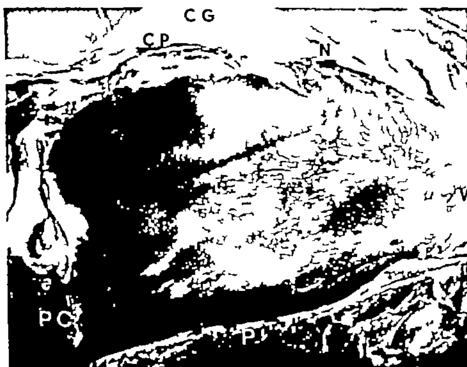


Fig 3 Nasal Septum. Human fetus 22 weeks. As seen in ethanol after immersion in 1.5% osmic acid. Shows extent of the olfactory epithelium in the upper half of the nasal mucosa on the septum. Note the regularity of the 'olfactory margin' and the thickness f the epithelium relative to that of the adjoining respiratory areas. Respiratory epithelium shows numerous openings of ducts of nasal glar ls *CG* crista galli *N* nasal bone; *P* hard pal e *V* nasal vestibule; *PC* region of the posterior ch e nae.



Fig 4 Illustration representing the ultimate ppearance of the olfactory area on the nasa septum of woman 32 years of age as seen in ethanol after immersion in 1% osmic acid. The olfactory margin displays characteristic irregularity often associated with regression atrophy of the olfactory sense or gan (epithelium) that commonly occurs with ge. Islets of ciliated respiratory tissue are seen within the olfactory area.

formly and consistently black with a charac teristic velvet sheen while the adjoining *respiratory epithelium* appears purple, violet or pur ple-blue The various colour changes observed depend on the angle of incidence of light falling onto the mucosal surface The boundary line or *olfactory margin* appears clean-cut and clearly defined.

Furthermore, a topographical perception of the thickness of the olfactory epithelium relative to that of the adjoining respiratory areas is obtained (Figs ? 3)

In young guinea pigs, rats and rabbits the olfactory margin is generally regular This is true of man initially (Fig. 3) but early in life (1–2 years) alterations occur and impart to it a characteristic irregularity This early change in the regular contour of the olfactory



Fig 5 Photograph of part of the nasal mucosa on the nasal septum in the olfactory region of a woman 25 years of age taken with the specimen immersed in ethanol following its treatment with 1% osmic acid. It shows clearly the characteristic irregularity of the olfactory margin A mixture of ciliated (white) and olfactory (dark) tissues is seen within the left half of the specimen. *CP* cribriform plate *CRE*, ciliated respiratory epithelium.

in man with age is greatly exaggerated in later life (Figs, 4 5 6)

Whether the demarcation line between the olfactory and the respiratory areas is regular or irregular the junction between the two territories as seen in histological sections is always abrupt (Figs. 7 8, 9) Such junctions show no intermixing of cell types Only under certain circumstances such as with advanced age or disease can a mixture of the two tissues be observed within the olfactory area (Figs, 4 5 6)

Significance

Supposedly normal morphological descriptions are often those of epithelia showing degeneration or disease Appearances of epithelia so altered vary and so do their descriptions This



Fig 6. Photograph of a specimen similarly treated as that shown in Fig. 5 obtained from the anterior half of the olfactory region of a man 45 years of age. The olfactory margin is highly irregular above and is arrowed below where it appears less conspicuous. There is also great admixture of ciliated and olfactory tissue which gives the olfactory region a bizarre and characteristic topography The ciliated areas appear higher and are histologically thicker than the surrounding parent olfactory epithelium. Arrows indicate olfactory margin *CP* cribriform plate *CRE*, ciliated respiratory epithelium.

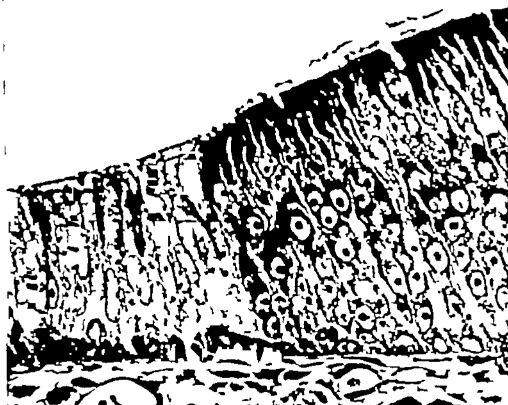


Fig 7 Phase contrast micrograph f the olfacto-respiratory junction Guinea pig. Section obtained from the olfactory margin on dorsum of endoturbinal 1 f specimen show in Fig. 2. Note thickness of the olfactory epithelium and conspicuous sensory cell nuclei. The olfactory epithelium slopes down to meet the respiratory epithelium at the junction which is abrupt. 760

invariably leads to conflict and controversy In the absence of macroscopic parameters of normality detailed histological and ultrastructural descriptions can confuse rather than illuminate issues

The macroscopic method described above is useful not only in the identification and precise localisation of the olfactory epithelium in man and the experimental mammal but also in its preservation for examination by light and electron microscopy Further the method may be applied to a study of the pathologically altered nasal mucosa of man.

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ZUSAMMENFASSUNG

Untersuchung in Ethanol on fixierten Praparaten der nasalen Mucosa in auffallendem Licht zeigt die genaue Lokalisation des olfaktorischen Epithelium beim Menschen und bei anderen Mammalien Es wird gezeigt, dass die olfaktorische Kante beim Menschen egelmassig ist Mit ungefähr 2 Jahren beginnt die Kante eine unregelmässige Form anzunehmen, wenn die Degenerat des olfaktorischen O (Epithelium) einsetzt.



Fig. 8 Phase contrast micrograph of the olfacto-respiratory junction. Human adult. Section obtained from the olfactory margin of the fixed specimen of nasal mucosa shown in Fig. 5. Note the abruptness of the junctional tissue. At least four olfactory vesicles (arrow) can be identified on the surface of the olfactory epithelium to left of junction. 1000.



Fig. 9 Phase contrast micrograph of section similar to that shown in Fig. 8 obtained from the margin of a ciliated islet from specimen shown in Fig. 6 again showing an abrupt junction (split) of ciliated islet and parent olfactory epithelium. The split shown may indicate junctional plane of tissue weakness or diminished resistance that may exist at olfactory margins of nasal mucosae so altered by age or disease. Arrows indicate olfactory vesicles. 1000.

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EXPERIMENTAL INVESTIGATIONS ON THE PENETRATION OF ^{198}AU FROM NASAL MUCOUS MEMBRANE INTO CEREBROSPINAL FLUID

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(Received November 10 1969)

Abstract Determinations of the activity of the cerebrospinal fluid in 32 rabbits were made after administering the isotope ^{198}A under the mucous membrane of the olfactory region of the nose. Such activity indicates the direct penetration of ^{198}Au from this membrane into the cerebrospinal fluid of the anterior cranial fossa.

Numerous experimental works (Riser 1929 Yoffey 1949 1958 Bakay 1956 Seki 1964) have shown that dyes introduced into the subarachnoid space of the brain pass into the nasal mucous membrane and into the lymphatic system of the neck. Orosz and collaborators (1957) also showed that ^{32}P solution and radioactive thorium B pass directly into the cerebrospinal fluid and the central nervous system when these isotopes are injected under the mucous membrane of the olfactory region of the nose in rabbits and rats.

The aim of the investigations presented here was to study the possible penetration of radioactive colloidal gold (^{198}Au) from the mucous membrane of the olfactory region of the rabbit nose into the cerebrospinal fluid of the subarachnoid space of the anterior part of the brain

MATERIAL AND METHODS

Experiments were carried out on 32 rabbits which were divided into three groups. group I ^{198}Au was injected under the mucous membrane of the nasal olfactory region of these animals group II the radiocolloid ^{198}Au was injected into the marginal vein of the aural concha group III the radiocolloid ^{198}Au was injected under the mucous membrane of the olfactory region of the nose immediately after bilateral ligation of the common carotid artery

A suspension of colloidal ^{198}Au, radioactivity 100 μCi was injected unilaterally under the mucous membrane of the roof of the nose in the animals of groups I and III group II animals received an intravenous injection of the radiocolloid ^{198}Au with an activity of 300 μCi. The activity of the cerebrospinal fluid and the plasma of these animals was determined by a means of a Geiger–Müller window detector

RESULTS

First group of experiments

After local injection of the radiocolloid ^{198}Au under the mucous membrane of the olfactory region of the nose, in all the animals of this group the greatest concentration of the isotope was found in the cerebrospinal fluid taken from the cribriform plate and the base of the olfactory lobes of the brain. The mean activity of cerebrospinal fluid taken from the cribriform plate 1 hour after administration of the isotope was approximately twice as high as the activity of that taken from the base of the olfactory lobes and about four times higher than that from the base of the cerebral frontal lobes.

Two hours after the administration of the isotope the mean activity of the cerebrospinal fluid from the cribriform plate was approximately one-and-a-half times higher than in that taken from the base of the olfactory lobes and about seven times greater than in that taken from the base of the frontal lobes.

The activity of the cerebrospinal fluid removed after 1 hour from the cistern of the corpus callosum was, in most of the animals, somewhat lower than in that taken from the cribriform plate, and when taken from the corpus callosum cistern 2 hours after administration of the isotope, it was about one-and-a-half times lower than in that taken from the cribriform plate.

The mean activity of the cerebrospinal fluid taken from the cistern of the chiasma 1 hour after administration of the isotope was seven times lower than that taken from the cribriform plate region. Two hours after administration of the isotope the activity of the cerebrospinal fluid from the cistern of the chiasma was more than eight times lower than that from the cribriform plate. Very low activity of the cerebrospinal fluid from the cerebellomedullary cistern was noted.

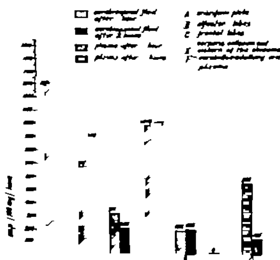


Fig 1 Comparison of the activity of the cerebrospinal fluid and plasma removed after 1 and 2 hours after administration of ¹⁹⁸Au.

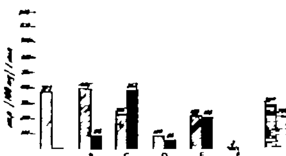


Fig 2 Comparison of the activity of the cerebrospinal fluid and plasma after intravenous administration of ¹⁹⁸Au.

Second group of experiments

After intravenous administration of the radio colloid ¹⁹⁸Au, it was found that in all the animals of this group ¹⁹⁸Au passes from the blood circulation into the cerebrospinal fluid of the cisterns of the subarachnoid space at the base of the brain both 1 and 2 hours after administration of the isotope The activity of the cerebrospinal fluid from the cribriform plate and the base of the olfactory lobes was a little higher than the activity of the plasma 1 hour after administration of the isotope On the other hand, 2 hours after isotope administration, the activity of cerebrospinal fluid from the cribriform plate and the base of the olfactory lobes of the brain was found to be low Cerebrospinal fluid activity when taken from the base of the frontal lobes 2 hours after injection of the isotope, was slightly higher than when taken from the base of the frontal lobes *1 hour after administration. The lowest radio*-activity only a trace, was observed in cerebrospinal fluid taken from the cerebello-medullary cistern 1 and 2 hours after intravenous isotope injection.

The third group of experiments

One hour after local injection of ¹⁹⁸Au together with bilateral ligation of the common carotid artery a very high activity in the cerebrospinal fluid from the subarachnoid space of the anterior cranial fossa was noted in all animals. The activity of the plasma was minimal. Cerebrospinal fluid activity when taken from the

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THE MALIGNANCY OF MIXED TUMORS OF THE PAROTID GLAND

A Clinicopathological Analysis of 70 Cases

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Abstract Sixty-four benign mixed tumors and car cinoma in mixed tumor cases were treated in this hospital in 1952 1964. The material was reclassified from a total of 119 various parotid tumors treated. The histopathology was correlated with the clinical history mode of treatment, 5-year follow-up and the number of recurrences. The criteria set forth by Eneroth and colleagues for the diagnosis of car cinoma in pleomorphic adenoma were strictly followed in the classification of mixed tumors. The results showed that only these behaved in a malignant fashion and the rest of mixed tumors showed a benign course of disease. The number of recurrences was higher in the group of tumors with multiple nodules, otherwise no differences in the behaviour was noted as related to cellularity cell atypism and mitotic Of the 6 patients with carcinoma in tumor three had received irradiation therapy for a parotid tumor not surgically removed 8–4 years prior to present rapid enlargement. In a group of patients with a comparably long history of parotid enlargement due to benign mixed tumor none was similarly treated before.

Tumors of the parotid gland form a challenging group of tumors, the classification of which has posed considerable difficulties for pathologist through the years. This seems to be due mainly to the relative scarcity of tumors seen by any individual examiner as well as the necessarily refined and rather recently developed surgical techniques required in the handling of the anatomical area. Thus apparently the first larger parotid tumor material compiled was that published by Ahlbom in 1935 as an extensive monograph. Only as late as 1954 the large retrospective material by Foote & Frazell did appear which has set forth the main criteria used in the classification of salivary gland tumors.

Particularly as concerns mixed tumors, which form a large majority of salivary gland neoplasms, has there been great uncertainty concerning the correlation of the histopathological findings and clinical behaviour. Apparently stemming from Ahlbom's monograph and appearing in subsequent papers, an ill defined label of "semi-malignancy" has been frequently attached to mixed tumors on the basis of such histological criteria as increased cellularity cylindromatous structures and incomplete encapsulation. The investigators at the Institute of Radiopathology at Karolinska Institutet in Stockholm have attacked this particular problem on the basis of a large retrospective material of salivary gland tumors (see Eneroth, 1964). They conclude that the mixed tumor (pleomorphic adenoma as they prefer to call it) is an entirely benign tumor and that the criteria mentioned above and previously associated with malignant potential or semimalignancy did not correlate with an increased propensity for tumor recurrences or clinically malignant behaviour. The few cases (1 6 %) which proved to be malignant (Moberger & Eneroth, 1968) were found to possess



Fig. 7. Serial sections of the course of the aqueduct (shown in Fig. 6) through bone towards the meningeal opening. On the left, the longitudinal aqueduct lumen (*A*) contains an erythrocyte mass also seen in the scala tympani (*ST*). The inferior cochlear vein (*V*) runs under the scala tympani roof and there is also a cross-section somewhat further away. In the middle, in section 375 μ apart, the aqueduct lumen (*A*) has just emerged from bone on one side and is filled with erythrocytes. The cochlear vein transverse lumen (*V*) is seen further off the scala tympani. On the right, in a section 600 μ apart, the aqueduct opening into the subarachnoid space is distinct and filled with a mass of erythrocytes. The cochlear vein (*V*) is also totally outside the bone and is here situated 1.5 mm away from the aqueduct. × 12.

480 μ. In this area there were two distinct para-canals in the aqueduct. The aqueduct emerged from the bone at a point 3.5 mm from the scala tympani, the vein was seen on one side, the other side was surrounded by dura with dense extensions into the funnel. The loose arachnoid tissue appeared in close proximity to the dura (Fig. 10). Further sections showed the vein to lie side by side with the aqueduct without intervening bone, and here the aqueduct lumen was first seen to contain neural tissue. The main mass of the glossopharyngeal nerve then became more and more prominent at the point where the aqueduct funnel was in contact with the subarachnoid space.

Case 3, left ear

The aqueduct was sectioned across its slope. The scala tympani orifice had a width of 300 μ and was nearly devoid of soft tissues. Near the scala tympani end, the 4 mm long aqueduct cross-sections measured about 150–300 μ and the lumen widened steadily towards the external orifice. The vein canal had roughly similar width at the scala tympani. It was separated from the aqueduct by 300 μ and the distance slowly increased to 900 μ (Fig. 11). In several areas the aqueduct lumen was connected with the neighbouring bone by short paracanals filled with dural tissue. The aqueduct lumen showed a distinct arachnoid mesh



Fig 10 A section 350 μ part from that shown in Fig. 9 The aqueduct (*A*) has just emerged from bone and is lined with thick dural tissue (*D*) adjoining the glossopharyngeal nerve. The width of the aqueduct is 600 900 μ The tubular dural lining is partly detached from the bony wall, and the arachnoid mesh (*Ar*) with a grey precipitate (*P*) inside has collapsed on to the right side of the aqueduct lumen. The tubular dural space for the aqueduct was w ll maintained throughout the meningeal opening. 100.

filled with loosely textured arachnoid mesh. In case 2, in which death resulted from cere bral hemorrhage the arachnoid mesh was filled with erythrocytes which had migrated into the base of the scala tympani. This clearly shows that the aqueduct, even if filled with arachnoid mesh, allows easy passage, not only of fluids but also of corpuscles the size of human erythrocytes. These data are in agreement with the findings of Crowe (1930) and of Holden & Schuknecht (1968) of whom the latter investigators demonstrated red blood cells in the scala tympani in 6 ears out of 12 in 6 adult subjects who had died of cerebral hemor rhage.

In none of the infant ears was there a bar rier membrane across the external orifice of the aqueduct. The arachnoid mesh seen in the aqueduct lumen gradually disappeared at the internal orifice, merging with the thin endothelial lining of scala tympani. In our series of adults (Palva & Dammert, 1969) 2 out of 20 ears showed a structure resembling a barrier membrane these new data suggest that it must be rather rare Furthermore it is unlikely that this membrane" is impermeable and intact, it is more likely to be simply a continuation of the arachnoid mesh of the aqueduct lumen, allowing a similar transport of fluid and cor puscles as the canal mesh itself

In some sectional planes the aqueduct emerged rather abruptly from the bone on the meningeal side and there were no large funnels, but if the section was made in the plane of the aqueduct and the internal acoustic mea tus, a distinct short funnel appeared In adult ears, all walls of the funnel seemed to be longer The point of emergence was distinct and



Fig. 11. Case 3, left ear, transverse sections of aqueduct (*A*) and vein (*V*). The section shows (× 35) the vein measuring 300 × 590 μ whereas the aqueduct has a width of 560 × 900 μ and shows a distinct paracanal of 450 μ into the neighbouring bone. The aqueduct and vein are separated by 900 μ.

Fig. 12. The meningeal opening of the aqueduct in case 3, left ear. This section is 0.7 mm away from that in Fig. 11: the aqueduct (*A*) lumen is just outside the bone, whereas the vein (*V*) is still within the bone. The thick dural lining forming the tubular soft wall of the aqueduct is thick particularly in the inferior part of the aqueduct. The distance between the aqueduct and vein is 750 μ. × 40.

the tubular dural lining of the aqueduct retained its lumen even in the mass of glossopharyngeal nerve adjoining the jugular bulb side of the aqueduct. Thus there was a clear connection with the subarachnoid space.

The soft tissues around the meningeal orifice of the aqueduct could be preserved in all specimens. This soft tissue, dura, arachnoid and the glossopharyngeal nerve, is so abundant that it is understandable that infectious processes, as suggested by Crowe (1930) may become sealed off from the aqueduct and cochlea by simple soft tissue swelling. On the other hand, particularly in infants and children with wide and short aqueducts and large-meshed arachnoid network, purulent infection from the subarachnoid space may penetrate into the cochlea during childhood meningitis. In adults, the longer and relatively more narrow canal with its arachnoid mesh apparently is better protected by soft tissue swelling at the external orifice of the aqueduct and in the canal itself.

A thorough search was made for the corpora amylacea earlier reported to occur sometimes in abundance, round the meningeal opening of the cochlear aqueduct of adult temporal bones.

None was found in the sections of the present series, a fact which accords well with the data of Karlefors (1924) Meurman (1930) and Waltner (1947) This also substantiates our view that these formations are products of degeneration and contraction of arachnoid cells and fibres which are infiltrated by calcium salts and protein precipitates. The exact age at which corpora amylacea begin to form is uncertain Meurman's youngest case was reported to be 7 years and Waltner's, 3 years. In the latter case there were only few (one or two) corpora amylacea and they were seen in increasing numbers after the age of 7

The soft tissues at the meningeal opening as well as the arachnoid mesh in the lumen itself obviously hinder a direct fluid flow from the CSF-space into the perilymph, and the exchange of fluids between the CSF-space and perilymph apparently occurs slowly and by seepage The fact that such an exchange of fluid occurs, apparently both through the aqueduct and along the eighth nerve fibres, has been documented in several reports on the composition of cerebrospinal fluid and perilymph (Palva & Raunio 1967 1968 1969)

ZUSAMMENFASSUNG

Der Aquaeductus Cochleae hatte in 6 Temporalknochen von Neugeborenen eine Mittelwertlänge von 3,5 mm, die Hälfte vom Mittelwert bei Erwachsenen (6,2 mm). Seine Diameter war am engsten 0,5–1 mm von der cochlearen Mündung entfernt, doch auch da relativ gross, wenigstens 150 μ. Der Gang hatte eine dünne Durabekleidung am Knochen mit einem lokkeren Arachnoidalnetz, das die Erythrozyten ins *Scala Tympani wandern liess. Am cochlearen Ende* wurde keine membranartige Struktur gefunden am meningealen Ende wurde das Lumen von der tubulären Dura auch ohne Knochenstütze bewahrt und die Öffnung geschah in den Subarachnoidalraum neben dem N. Glossopharyngeus nahe der Bulbus jugulare. Keine Corpora Amylacea wurde gefunden.

Das Arachnoidalnetz hindert nicht die Zirkulation zwischen Zerebrospinalflüssigkeit und Perilymphe die relative Weite des Kanals macht die Häufigkeit von Innenohrenbeschädigungen bei Kindermeningitiden verständlich. Die Protektion kann bei einer infektiösen Schwellung der Weichteile bei der Kanalmündung und dem Kanal selbst stattfinden.

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BONY FIXATION OF THE MALLEUS AND INCUS

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Abstract A material of 12 patients with bony fixations of the malleus and incus in the attic is presented. Of these patients 9 had intact, 3 interrupted ossicular chain. Various types of bony fixation of the attic were found. Isolated fixation of the incus, isolated fixation of the malleus, bony fixation of the malleus as well as the incus and ossification of the ligaments. Treatment consisted in removal of the new-formed bone with mobilisation of the ossicular chain which was preserved intact. The results were good. Among the tympanoplasties in which the ossicular chain was intact, it was fixed in the attic in 10% of the cases, and among those in which the ossicular chain was interrupted in only 1.2% In most cases the cause was chronic otitis, in one case congenital anomaly The aetiology and pathogenesis are discussed. It is concluded that post-infectious bony fixation presumably arises during the healing of localized osteitis. Other aetiological possibilities—such as increased pneumatization in the attic, high situation of the malleus, trauma and otosclerosis—are described and discussed.

Postinflammatory bony fixation of the auditory ossicles in the attic was mentioned as early as 1908 by Politzer Due to the marked advances in otosclerosis surgery and of microsurgery in chronic otitis, fixation of the ossicles in the attic has again come into the focus of interest. Beck (1960) described 3 cases of bony fixation of the incus and malleus in the attic found following tympanoplasty in Zöllner's clinic. Goodhill (1960) reported one case of bony fixation of the incus which he assigned, together with several other conditions suggestive of otosclerosis, to a group which he named pseudo-otosclerosis. House (1963) mentioned fixation of the malleus as a complication to surgery on the stapes, and Guilford (1963) pointed out that the mobility of the malleus should always be investigated before removing the stapes. Moreover the mobility of the malleus and incus should preferably be investigated before myringoplasty and tympanoplasty with intact ossicular chain.

During the past 4 years we have systematically investigated whether the malleus and incus were fixed in the attic before embarking upon tympanoplastic operations. In suspicious cases we have opened the attic. The object of the present study was to establish the frequency of the various types of bony fixation in the attic and to present the results of treatment. It is not unlikely that failure to obtain hearing improvement after well-accomplished myringoplasty may be due in some cases to bony fixation of the malleus or incus in the attic. In particular there is a possibility that well-accomplished mastoidectomy including tympanoplasty which has resulted in satisfactory hearing improvement may later be followed by new formation of bone and fixation of the malleus or incus in the attic, again aggravating the hearing.

MATERIAL

Among patients undergoing tympanoplasty we found 9 cases in which the malleus and/or incus showed bony fixation in the attic. It was common to all these patients that for several

Table I *Material types of bony fixation and treatment*

NO	AGE SEX	PREVIOUS OTITIS	PREVIOUS SURGERY	MIDDLE EAR MUCOSA	EPITYMPANIC MUCOSA	PERFORATION OF DRUM AND OSSEOUS FIXATION	TYMPANO PLASTY
	8 ♂	3 ACUTE		NORMAL, EXOSTOSIS	ORMAL		I ANNULUS EMOVE
2	9 ♂	FOR YEARS RECURRENT, SEROUS SEVERAL ACUTE	3×TUBULATION	ORMAL	ORMAL		I ANNULUS NARROWER, PRESERVED
3	3 ♂	CHRONIC FROM AGE YEAR DRY FOR 8 MO	MASTOIDECTOMY AT 7 YEARS	SLIGHTLY THICKENED	NORMAL		I ASCIA ANNULUS NARROWED PRESERVED
	25 ♀	CHRONIC FROM CHILDHOOD DRY FOR MO		NORMAL, PERISTAPEDIAL ADHESIONS	NORMAL, FIBROUS ADHESIONS		I AND STAPEDI-OLYSIS, [illegible] ANNULUS PRESERVED
5	28 ♀	CHRONIC FOR YEARS DRY FOR YR	MYRINGOPLASTY 11 YEARS AGO	THICKENED, PERISTAPEDIAL ADHESIONS	THICKENED FIBROUS ADHESIONS		I SKIN ANNULUS PRESERVED
6	37 ♀	CHRONIC IN CHILDHOOD DRY MANY YEARS		NORMAL	NORMAL		FASCIA ANNULUS REMOVED
	53 ♀	CHRONIC FOR MANY YEARS DISCHARGE		GRANULATING	GRANULATING		I ASCIA ANNULUS REMOVED MASTOIDECTOMY
	54 ♀	CHRONIC FROM CHILDHOOD DRY FOR YR		NORMAL	NORMAL, FIBROUS ADHESIONS		I FASCIA ANNULUS PRESERVED
	80 ♂	CHRONIC O MANY YEARS DRY FOR SEVERAL YRS		NORMAL PERISTAPEDIAL ADHESIONS	NORMAL, CHOLESTEATOMA		II ASCIA INTERPOSITION OF INCUS ANNULUS REMOVE MASTOIDECTOMY

years they had had hearing impairment of the conductive type between 45 and 60 dB and that the ossicular chain was intact, but fixed

Seven had a long history of chronic otitis with recurrent discharge (Table I) However the ears had been dry for quite a long time before the operation in all cases but one (case 7) who still had active chronic otitis with discharge Five of the patients had large perforations of the pars tensa, 2 of the pars flaccida. Two patients had previously had operations, one of them (case 5) simple mastoidectomy and the other one myringoplasty without any effect upon the hearing.

Two patients had not had chronic otitis and did not present any perforation of the drum at the time of the operation One of them (case 1) had only had 3 episodes of acute otitis, but before the first episod. his hearing had been considerably impaired Through the drum a reddish, tumour-like mass could be seen, issuing from the posterior wall of the middle ear The other patient (case 2) had a history of several attacks of acute otitis during infancy and for 2 years recurrent serous otitis in both ears, treated 3 times with tubulation and politzerization. The hearing had always improved in the contralateral ear never in the ear which later showed bony fixation. The Eustachian tube was patent in all 9 cases.

On preoperative microscopic examination of the drum, the handle of the malleus or the in-

cus were found to be immovable on palpation with a stiff probe, and no hearing improvement was obtained by covering the perforation by a paper prosthesis. Thus, in most cases the diagnosis could be made before the operation. Accordingly the tympanoplasty was done by the retroauricular or Heermann's approach, opening the attic and the middle ear. At operation the middle-ear mucosa was found to be slightly thickened in 2 cases (Table I), granulating in one, while in the remaining cases it was of normal appearance. In 3 cases there were fibrous adhesions around the stapes, in one of these cases (case 4) also degeneration of the peristapedial ligament, so that stapediolysis had to be performed.

The attic was in 7 cases of normal appearance, in one case thickened and in one case granulating. In 3 instances there were a few loose fibrous adhesions.

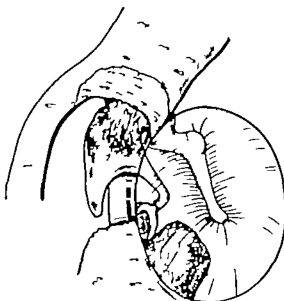


Fig. 1. Isolated bony fixation of the incus as congenital anomaly (case 1). The body of the incus is fixed by wide, smooth-walled, compact bridge issuing from the bony annulus. From the posterior wall of the middle ear a large exostosis is seen to arise.

Types of Bony Fixation

Several types of bony fixation in the attic were found. (1) Isolated fixation of the incus. (2) Isolated fixation of the malleus. (3) Fixation of the incus and malleus. (4) Ossification of the ligaments.

1. *Isolated bony fixation of the incus* was found in 2 cases. In the first one (Table I, case 1) the lateral surface of the incudal body had completely fused with the bony annulus through a wide, smooth-walled, compact bony bridge lined with normal mucosa (incus-annulus fusion). The incus mucosa proceeded along the bridge to the annulus. This patient also had a large exostosis arising from the posterior wall of the middle ear (Fig. 1). This exostosis had no connection to the incudo-stapedial joint or to the pyramidal eminence. As the hearing impairment and the exostosis had been observed at the age of 4 years, and as the patient did not have any history of otitis, the *incus-annulus fusion* as well as the exostosis in the middle ear may presumably be interpreted as a congenital anomaly.

In the other case of isolated incus fixation (case 3) there was a bony bridge between the lateral aspect of the incus and the bony annulus. However this connection was rather irregular extending also to the superior surface of the incudal body. The irregular nodular bridge was lined with normal mucosa.

2. *Isolated fixation of the malleus* was found in 2 cases (cases 4 and 5). From the anterosuperior attic wall a wide bony bridge extended right to the head of the malleus which it thus fixed. Although the malleus showed no bony fusion with the exostosis, very short, tight, and thick adhesions extended from the exostosis to the malleus (Fig. 2).

3. *Bony fixation of the malleus and incus* was found in 4 cases. In all these cases the malleus showed bony fusion with the anterosuperior attic wall by a wide, irregular bony bridge lined with mucosa (malleus-attic wall fusion) (Fig. 3). In one case it had also fused with the medial attic wall. In 2 cases (cases 6 and 9) the incus had fused with the annulus (incus-annulus fusion) and in 2 cases (cases 2 and 7) with the superior attic wall (incus-attic wall fusion) (Fig. 4).

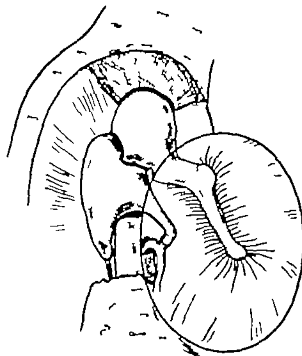


Fig 2 Isolated fixation of the malleus. From the antero-superior attic wall a wide bony bridge extends right to the head of the malleus.

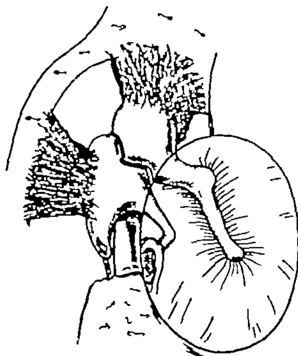


Fig 4 Bony fixation of the incus and malleus. The incus is fixed by a bony bridge to the superior the malleus to the antero-superior attic wall.

4 *Ossification of the ligaments* In one case (case 8) the incus and malleus were fixed due o ossification of the anterior and superior ligm of the malleus as well as the posterior of the incus. These ligaments were



Fig 3 Anterior part of the attic in case 6. Between the head of the malleus (*M*) and the antero-superior attic wall a wide bony bridge (*O*). The anterior malleal ligament (*L*) is intact. The incus (*I*) with the incudo-malleal joint is visible.

converted into thick bony bridges lined with mucosa.

Another 3 cases of bony fixation were found among patients undergoing operation for long-lasting, chronic otitis However in these cases *the ossicular chain was interrupted* and the fixation had no influence upon the hearing impairment: in one, the long process of the incus was absent, while in the other two both the long process of the incus and the stapes were absent. In one case there was isolated fixation of the incus which was fixed in two sites (Fig. 5) from its superior surface an irregular bony bridge extended to the superior attic wall (*incus-attic wall fusion*) and from its *medial* surface another bridge extended to the lateral semicircular canal (incus-semicircular canal fusion) In the other case the malleus was fixed by a wide bridge to the antero-superior attic wall (malleus-attic wall fusion) and the incus to the semicircular canal (incus-semicircular canal fusion). Ten years previously this patient had undergone simple mastoidectomy and 2

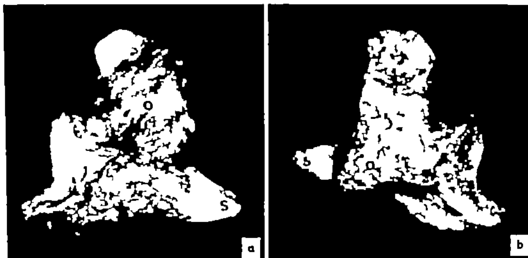


Fig. 5. Bony fixation of the incus in a patient in whom the long process of the incus is absent. (*a*) From the superior surface of the incus body an irregular bony bridge (*O*) extends, fixing the incus to the superior attic wall. Postinflammatory changes and sequelae of osteitis with erosion of the surface of the body and short process (*S*) are evident. (*b*) From the medial surface of the body there extends the new bone (*O*) which fixes the incus to the lateral semicircular canal. This bone is irregular with numerous small depressions and canals.

years previously attico-antrotomy. In the third case the malleus was also fixed to the antero-superior attic wall, but the incus was fixed in two sites: Its superior surface was fixed to the attic wall (incus-attic wall fusion) and its medial surface to the lateral semicircular canal (incus-semicircular canal fusion). The fixation was so firm that on removal of the incus a fistula arose on the lateral semicircular canal.

TREATMENT AND RESULTS

In all cases the newformed bone was first detached from the ossicular chain by a burr. Thereafter the newformed bone could easily be removed from the wall, and thereby the ossicular chain was mobilized. To avoid damage to the cochlea, the drilling was done very gently with the finest burr. The attic wall was thinned, and the distance between the ossicles and the wall made as wide as possible. The mucous membrane was spared as far as possible. In all cases the bony annulus was narrowed, and in half the cases removed in a small area at the site overlying the incus (Table I). In one case (case 9) the incus was removed because of a cholesteatoma extending below it, and tympanoplasty II was done with interposition of the incus. In the remaining 8 cases the ossicular chain was preserved intact. The perforation of the drum and of the attic membrane was in most cases closed with fascia.

In the 3 cases where the ossicular chain was already interrupted, tympanoplasty II with interposition of the incus was done in one case and tympanoplasty III in two.

The *results* were assessed by the condition 2–3 months after the operation tested for the frequencies 500–2 000 c.p.s. (Fig. 6). All patients had obtained an average hearing gain of more than 10 dB, eight of more than 20 dB. Eight out of the 9 patients with intact ossicular chain obtained a hearing of 30 dB or more. The average preoperative hearing in the 500–2 000 c.p.s. range had been 49 dB, postoperatively 26 dB. The average hearing gain in the 9 patients with intact ossicular chain was 23 dB.

Fig. 6 illustrates the pre- and postoperative hearing on the basis of speech audiometry

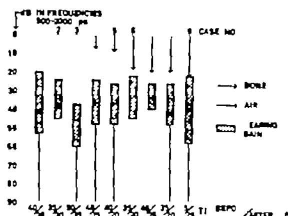


Fig 6 Results –3 months after the operation in the 9 patients with intact ossicular chain. The preoperative air and bone conduction, the hearing improvement in the 500–2 000 c p s range, as well as the pre- and postoperative TI are given.

(TI = threshold of intelligibility) After the operation the TI had improved in all cases, as a rule by 25 dB or more

Seven patients could be examined again 1 year after the operation. In 4 the hearing was unchanged in relation to the findings 2–3 months after the operation, while in 3 the average hearing had improved by 5–10 dB as compared with the status 2–3 months after operation, indicating that re-fixation of the ossicles had not taken place

Our therapeutic principle has been to open and extend the attic, to remove newformed bone, and to preserve the ossicular chain intact. Goodhill (1966) Guilford & Anson (1967) as well as Powers et al. (1967) prefer a by-pass technique removal of the incus, resection and extraction of the malleal head, and if the stapes is mobile tympanoplasty II with interposition of the incus. When the stapes is fixed by otosclerosis they perform stapedectomy and apply a steel-wire prosthesis from the handle of the malleus to the foramen ovale As their reasons for using this technique, they adduce the risk of re-fixation of the malleus and possible damage to the cochlea in drilling the ossicles. When the attic wall is thinned, and thus the distance to the malleus is increased, as in our procedure, there is little likelihood of bony re-fixation. In our follow up examinations we have not found any signs of re fixation of the ossicles and no perceptive hearing loss as a consequence of the drilling. Although the named authors have obtained good results by their methods, we feel that in cases with large perforations the ossicular chain ought to be preserved, as this facilitates the application of the drum graft.

Incidence

Among the 89 tympanoplasties in which the ossicular chain was *intact* there were 9 (10%) with bony fixation, while among 247 tympanoplasties in which the ossicular chain was *interrupted* there were only 3 (1.2%) Among 128 patients with *dry ears* there were 8 cases (6%) and among 208 patients with *discharging ears* 4 cases (2%) with bony fixation in the attic Within the entire material of past and present chronic otitis bony fixation was found in 3 6%

In histological studies of 116 temporal bones showing the sequelae of otitis and chronic adhesive otitis, Ojala (1953) found one case (0.9%) with bony fixation of the malleus in the attic.

Among 3991 stapedectomies Guilford & Anson (1967) found 34 cases (0 9%) of bony fixation of the malleus, none with fixation of the incus. Of these patients 22 also had stapedial otosclerosis. Guilford & Anson were surprised that their entire material of tympanoplasties and of active chronic otitis included only 3 cases of fixed incus and one of fixed malleus. Among their primary stapedectomies Powers et al. (1967) found bony fixation of the malleus in 1 6% Their material comprised 35 cases, 27 of whom also exhibited stapedial otosclerosis

Sleeckx et al. (1967) among 2 000 primary stapedectomies, found 8 cases (0 4%) and among 200 re-operations 27 (13.5%) with bony fixation of the malleus and/or incus. Stapedial otosclerosis was present in all 35 cases. In another 18 cases showing bony fixation in the attic, otosclerosis was absent.

It is difficult to explain the marked differ

in incidence between the named mate-
ls and ours. On the one hand, we found of the incus and fixation of the malleus to be equally common, while fixation of the stapes was not observed in a single case. On the other hand, we had a high percentage of cases with bony fixation among tympanoplasties with intact ossicular chain. Part of the explanation of these differences may be that in every tympanoplasty we have endeavoured to preserve the ossicular chain intact, and in the event of the slightest suspicion of a reduced mobility of the malleus or incus we have invariably opened the attic.

Aetiology and Pathogenesis

Apparently bony fixation may be caused by several factors: (1) congenital anomaly (2) abnormally increased pneumatization in the attic, (3) abnormally high position of the malleal head, (4) infection (osteitis, otitis, cholesteatoma) (5) fracture of the temporal bone and indirect trauma, (6) surgical injury and possibly (7) otosclerosis.

1 The one case of isolated fixation of the incus (case 1) must be due to *congenital anomaly* as the patient had never had otitis and as the attic and middle-ear mucosa was entirely normal. Moreover there was an exostosis in the middle ear (Fig. 1) The bony bridge joining the incus to the bony annulus was smooth and compact unlike the other cases in which it was rough and irregular Bony fixation of the malleus and incus may also be a minor solitary anomaly with no other associated deformities (House, 1956) Andersen et al. (1962) have described 3 cases of congenital deformation of the malleus, in which the malleus was joined to the posterior meatal wall by a bony bridge These patients also exhibited negligible deformation of the auricle Among 53 cases of bony fixation of the ossicles, described by Sleeckx et al. (1967) a few had at the same time stapedial otosclerosis and minor congenital anomalies, such as membranous atresia of the meatus, dehiscence of the tympanic part of the facial canal, absence of stapedial tendon, and others In severe and multiple congenital deformities of the middle ear bony fixation of the malleus and incus is a common finding (Scheer 1967)

2 Goto et al (1968) reported on 3 cases of fixed malleus and 3 of isolated fixation of the short process of the incus, caused by air cell septa due to *abnormally increased pneumatization* The incus and malleus were surrounded by but not fused with, the septa. In a histological study on 40 temporal bones Ueda (1963) found one case of intense pneumatization in the attic and an air-cell septum fixing the incus. We had no such cases. In all our cases the bony bridge was wide and massive, in most cases fused with the incus or the malleus. Pneumatization was decreased in our cases.

In operations on normal temporal bones we have not infrequently found minor exostoses arising from the superior medial, or anterior attic wall. These exostoses reached in some cases as far as the incus or malleus which, however had invariably preserved their full mobility It is possible that attic exostoses are due to abnormal pneumatization in that event they have not been absorbed by the mucosa during the process of pneumatization.

In tympanoplasties for chronic otitis and its sequelae, where the attic has been opened, we have also seen minor exostoses in the attic. In some cases these exostoses have been joined by fibrous bands to the incus or malleus which, however have been normally mobile It is quite conceivable that these exostoses involve a risk of bony fixation, especially in otitis where they may lead to bony fusion.

3 *Abnormally high position of the malleal head* may according to Altmann (1965), predispose to bony fixation in the attic. Dietzel (1960) found an abnormally high position of the malleus in 10 out of 55 temporal bones with stapedial otosclerosis. Altmann found only one case among 42 temporal bones with otosclerosis. In this case, however there was no fixation of the stapes, but the head of the malleus was high and close to the roof of the

attic, but without touching it. In Altmann's opinion mechanical irritation between the malleal head and attic roof may later lead to bony fusion Among our cases of bony fixation the head of the malleus or the incus were not of an abnormal site, and the distance to the attic walls was normal.

The same situation as found when the malleal head is abnormally high-seated is created by an *abnormally low seated* attic wall. This we have observed in a few cases during operations on normal temporal bones and in tympanoplasties The distance between the ossicles and the attic wall is thereby reduced and the spatial relations narrowed. This might afford a risk of bony fixation, especially in the presence of infection

4 In the great majority of our cases the bony fixation must have been due to past *infection* Apart from the patient with congenital anomaly and another patient (case 2) with a history of numerous recurrences of acute otitis *during infancy all the patients had long-lasting* chronic otitis with large perforations of the drum In the cases reported by Beck (1960) and in that of Hilding (1965) the aetiology was also infection

) In post-infectious fixation the pathogenesis is easiest to explain in the case having ossification of the ligaments (case 8) As a consequence of the infection, the ligaments may undergo degeneration, followed by calcification and ossification. In the other post infectious cases it is more difficult to explain why new formation of bone should occur in this particular site In Beck's opinion, it is chronic irritation by the cholesteatoma which causes osteitic changes in the attic wall, malleus, and incus. However only one of our patients had cholesteatoma, the others having central perforation In my opinion, bony fixation must take place during the healing of chronic or acute otitis, as the majority of our patients had not had any discharge for a long time. Likewise, the ears have been dry in nearly all previously published cases. It is probable that severe infection may cause destruction of the attic mucosa and localized osteitis. During the healing of osteitis fibrous tissue may form between the ossicles and the attic wall, followed by calcification and ossification with bridging between the ossicles and the wall. Our 2 cases showing isolated fixation of the malleus (cases 4 and 5) in which the bony fusion to the malleus was not complete but only in the form of pronounced fibrous adhesions, might indicate that the ossification had stopped before reaching the malleus Fig. 5 clearly shows an amorphous, osteoid-like new formation of bone around the body of the incus and signs of osteitic erosion of its short process Another possibility is that the remnants of the effusion in the attic become delimited organized, and ossified in the course of healing

In tympanosclerosis the ossicles are very often fixed by tympanosclerotic masses, and yet bony fixation does not appear to bear any relation to the tympanosclerosis: None of our cases showed signs of tympanosclerosis, neither *in the drum, middle ear nor attic. Nor has* tympanosclerosis been described in previously reported cases of bony fixation (Ojala, 1953 Beck, 1960· Goodhill, 1966 Hilding, 1965 Guilford & Anson 1967 Powers et al 1967).

In chronic, adhesive otitis the malleus and incus are very often fixed in the attic, but this fixation is fibrous In our material there were no signs of adhesive otitis, and there was a striking paucity of fibrous adhesions in the *middle ear and attic. Moreover* the mucosa was in most cases of normal appearance Ojala has reported one case of bony fixation of the malleus in adhesive otitis occurring after calcification in the organized fibrous tissue.

5 During the healing process after *fracture of the petrous portion* of the temporal bone, formation of callus may fix the malleus or the incus. Does & Bottema (1965) found one case with bony fixation of the head of the malleus following fracture through the tympanic roof. In a material of 300 fractures of the temporal bone, not yet published, we found 35 cases in which a conductive hearing loss of 30–40 dB persisted for months after the trauma, al-

though the drum and tubal function were normal and the ossicular chain presumably intact. Possibly the hearing impairment in some of these cases has been due to bony fixation in the attic. As Elpern et al. (1965) in artificial fixation of the malleus, found an air–bone gap of 10–28 dB it is possible that post-traumatic fixation of the malleus or incus may be present more often than assumed so far in cases of post-traumatic conductive hearing loss.

Less violent, and indirect traumas, such as those of explosion, may in rare cases be responsible for bony fixation (Sleeckx et al., 1967).

6. *Surgical traumas* may also be imagined to cause bony fixation. During surgery on the attic the mucosa will be injured, and moreover small pieces of bone or dust from drilling as well as blood may remain in the attic and undergo organization and ossification during the process of healing. Among the 9 cases with intact ossicular chain we found one (case 3) with a history of simple mastoidectomy Among the 3 cases with interrupted ossicular chain there was also one who had a history of simple mastoidectomy as well as of attico-antrotomy Sleeckx et al. (1967) felt that surgical trauma must be an important aetiological factor in bony fixation, as they found bony fixation of the ossicles in the attic in 13.5% of their re-operations for otosclerosis.

7 As the largest number of patients with bony fixation of the malleus have been found in the presence of stapedial otosclerosis, Guilford (1963) felt that *otosclerotic activity in the temporal bone* was responsible for the bony fixation of the malleus. In microscopic studies of the excised malleal heads, however neither Guilford & Anson (1967), Powers et al. (1967), nor Sleeckx et al. (1967) could find any changes indicating malleal otosclerosis. Altmann (1965) in a histological study of 42 temporal bones with otosclerosis, also found no otosclerotic changes of the ossicles. In otosclerotic stapedial ankylosis, displacement of the footplate may occur and this causes upward displacement of the ossicular chain Thereby the head of the malleus and the incus will be brought closer to the attic walls and this will make for subsequent fixation, either due to constant mechanical irritation or to minor infections.

Although bony fixation in the attic was found to be considerably more common in our material than assumed previously and although otitis was the cause of bony fixation in the majority of our cases, infection *alone* cannot explain *all* the reported cases found in the course of operations for otosclerosis On the other hand, it is difficult to imagine that aetiological factors such as abnormal pneumatization, small exostoses in the attic, a high position of the malleal head and narrow space due to a low attic wall, or displacement of the ossicles by otosclerosis *alone* could cause bony fusion. However the named factors do afford a certain risk of fixation and when *combined with* acute otitis, they may cause bony fixation. In the presence of a swollen and thickened mucosa, the mechanical irritation between the malleus and the attic wall (Altmann, 1965) will be appreciably increased and may contribute to new formation of bone in otitis.

ZUSAMMENFASSUNG

Zwölf Patienten mit knöcherner Fixierung des Hammers und Amboss im Attik werden präsentiert. Bei 9 Patienten war die Gehörknöchelchenkette intakt, bei 3 unterbrochen. Es wurden verschiedene Typen der knöchernen Fixierung gefunden: Isolierte Fixierung des Amboss, isolierte Fixierung des Hammers, knöcherne Fixierung des Hammers und des Amboss und Verknöcherung der Ligamente. Bei der Behandlung wurde der neugebildete Knochen entfernt, die Kette remobilisiert und die Verbindung der Kette erhalten. Die Resultate waren gut. Bei Tympanoplastiken mit intakter Kette wurde knöcherne Fixierung im Attik bei 10% mit unterbrochener Kette bei 1,2% der Fälle gefunden. Ätiologi war bei den meisten Fällen chronische Otitis media, bei einem Fall kongenitale Missbildung Ätiologi und Pathogenese wird diskutiert. Es wird konkludiert, dass die postinfektiöse Fixierung wahrscheinlich während der Heilung eines lokalisierten Otitis entsteht. Andre ätiologischen Möglichkeiten, sowie vergrösserte Pneumatisation des Attik, hohe Lage des Hammers, Trauma und Otosklerose wurden beschrieben und diskutiert.

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THE CONNECTIVE TISSUE IN NORMAL AND OTOSCLEROTIC BONE

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Abstract. Normal and otosclerotic human stapes was studied by light and electron microscopy with view to disclosing generalized histologic changes in the stapes outside the otosclerotic focus that might lend support to the theory that otosclerosis is generalized connective-tissue disorder By means of various connective-tissue stains 43 stapedes, of which 31 presented an otosclerotic focus, were studied by light microscope eight stapedes, thereof four with otosclerosis, were examined by electron microscope. The structure of periosteum and bony tissue was found to be normal, and there were no significant differences in normal and otosclerotic stapes. Pre-otosclerotic changes were not disclosed in any preparation. The study provided no evidence of otosclerosis being generalized connective-tissue disorder

Among the theories that have been advanced about the aetiology and pathogenesis of otosclerosis one in particular has been much discussed during recent years. According to this theory otosclerosis is a generalized disorder of connective tissue which for unknown reasons is usually monosymptomatic.

The theory is supported by evidence of widespread connective tissue deficiencies that have been demonstrated at meticulous clinical examination of otosclerotic patients (Simson Hall, 1963 Arslan 1963) In 1962, Bentzen stressed the presence of an increased frequency of fractures, subluxations, subcutaneous haemorrhages, caries, and anomalies of hair and nails in patients suffering from otosclerosis In 1961 Bentzen & Stadil reported degenerative changes of the elastic and collagenous fibrils in skin biopsies from otosclerotics. Similar changes were found in patients with osteogenesis imperfecta, and it was concluded that there is a gradual transition from otosclerosis to osteogenesis imperfecta in the clinical manifestations of one generalized mesenchymal disorder Studies on the structure of the osseous connective tissue in these patients have not been published

Histology of the stapes

The finer structure of the stapes was described by Oesterle in 1933 by Bast & Anson in 1949 and by Altmann & Basek in 1961

The stapedial crura consist of two, sometimes three, layers of bone. The central layer of enchondral bony tissue is composed of mainly wavy fibrils running parallel to the longitudinal axis of the crus. The convex—and occasionally also the concave surfaces of the crura are covered with thin layers of periostal bony tissue with bundles of fibrils running at right angles to the longitudinally running fibrils.

The stapedial footplate consists of two layers of different embryologic origin: one cartilaginous layer facing the vestibulum and one enchondral bony layer facing the tympanic cavity The bony layer is sometimes very thin, particularly towards the major portion of the footplate, which frequently consists of cartilage only In the angle between the footplate and the crura the bony layer is thick, composed of haversian lamellar systems. The capitulum consists mainly of enchondral bone, more or less spongy in texture. The articular surface is covered with cartilage.

Reports on the ultrastructure of the temporal bone are rare. Chevance (1962) who was mainly concerned with undecalcified tissue, found the periodicity of the collagenic fibers to be 640 A. In 1968 Roydon & Smith electron microscopi studies of normal guinea-pig stapes and otosclerotic human stapes showed that the bundles of collagen fibrils outside the otosclerotic focus were spindle-shaped with an irregular tangled arrangement. Moreover the



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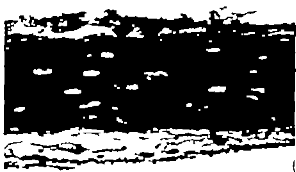


Fig 1 Bone structure in a normal stapedial capitulum. Collagenic fibres are red. Stain: van Gieson-Hansen, 130.

Fig 2 Bone structure in normal stapedial capitulum. All fibres except for the elastic membranes are coloured bright red. Stain: Sirius-red F_3BA, 260.

Fig 3 Anterior crus from otosclerotic stapes. Normal bone structure. Stain: Sirius-red F_3BA, 260.

Fig 4 Posterior crus from otosclerotic stapes. Dense structure of elastic fibres as in normal bone Silver staining (Bodian), 260.

ribbon of the collagen period was found to be broader than in normal collagen. Similar changes were present not only in the footplate, but also in the capitulum.

In 1965 Altmann, studying the auditory ossicles from otosclerotic patients, observed changes that could be interpreted as prestages or early stages of otosclerosis. Moreover biochemical changes have been demonstrated in early cases. Maurer showed, in 1962, that the connective tissue in the bones of otosclerotics contained lesser amounts of mineral and more protein than normal. He also found a lowered activity of alkaline phosphatase implying that the increased protein content originates in the intercellular substance, i.e. the fibrils, and not in the cells. Similar changes were reported by Soifer and co-workers in 1967 Significant biochemical alterations have not been found in the blood (Soifer et al., 1963) and the urinary excretion of amino acids and hexosamine was found to be normal in otosclerotic patients (Schoenheyder et al., 1966).

Personal Investigation

The aim of the present study was a histologic comparison of the structure of connective tissue of the bones in normal subjects and in patients suffering from otosclerosis. If otosclerosis is considered a generalized mesenchymal disorder the most obvious place to look for pre-otosclerotic changes must be that portion of the connective tissue which harbours the actual pathologic changes when the disorder becomes manifest.

MATERIAL AND METHODS

The investigation was carried out on the stapes as the most readily accessible material. Fifty-





Fig. 5 Osteocytes (A) Normal stapedial crura, × 6 860. (B) Otosclerotic crura. *n*, nucleus; *m* mitochondria *f* fibrils in the ground substance. No fibrils are visible. The mitochondria are partially disintegrated, presumably due to post-mortem changes.

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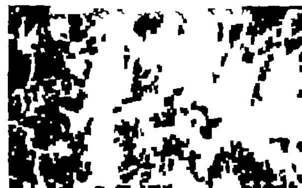
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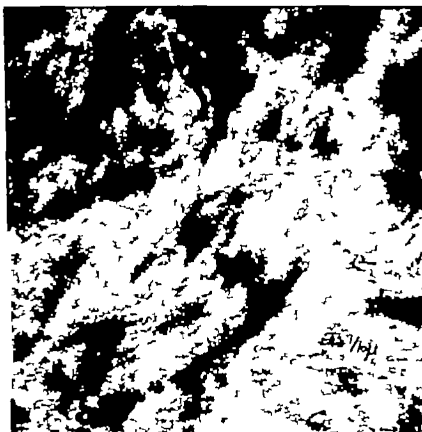


Fig. 7. Collagenic fibrils. The striation, consisting in one dark ribbon and two lighter ones, is clearly visible. The thickness of the fibrils is uniform in each fibril and from one fibril to another × 22 500.

one stapedes were examined, 35 of which had been removed at stapedectomy because of otosclerosis. 16 were removed at autopsy. Of the 51 stapedes 43 were examined under light microscope, 8 were studied by electron microscopy. Of 31 stapedes studied under light microscope 12 were normal.

All 43 stapedes were fixed in formalin, decalcified in formic acid and thereafter embedded in paraffin. The preparations were stained with haematoxylin–eosin and by the PAS-technique.

Collagen and reticular fibers were demonstrated by means of the van Gieson–Hansen technique, Weigert's iron haematoxylin and with Sirius-red (Sweat et al., 1964). Elastic fibrils were stained with silver (Boudain). Eight stapedes—four otosclerotic and four normal—were studied by means of a Phillips electron microscope.

After removal, the otosclerotic stapedes were cut into smaller pieces and fixed in 2.5% glutaraldehyde buffered with sodium cacodylate for 2 to 8 hours at 4 C. The specimens were washed in 0.2 M sucrose solution in cacodylate buffer and decalcified with EDTA adjusted to pH 7.2. After rinsing in sucrose cacodylate buffer and post-fixation in OsO_4 the specimens were dehydrated in a series of graded ethanol and embedded in Epon. Sections 1 μm in thickness stained with methylene blue were used for orientation. Thin sections were cut on a Reichert ultra-microtome, stained with lead citrate and studied under the electron microscope.

The four normal stapedes were fixed post mortem in 2.5% glutaraldehyde buffered with sodium cacodylate for 6 hours. Thereafter the specimens were cut to smaller pieces and fixed for an additional 4 hours. The subsequent procedure was identical to that described for otosclerotic stapes.



Fig 8 Collagenic fibrils in periosteum and otosclerotic bone. 7 600.

OBSERVATIONS

In evaluating the bony tissue we have applied the following parameter

1 The number and distribution of osteocytes (*as an indicator of the degree of autolysis* we noted the number of preserved nuclei)

2. The density distribution and course of the collagenic fibrils (the colour intensity was disregarded, since it varied considerably in either group of preparations)

3 The density distribution and course of the elastic fibrils.

4 The ultrastructure of the fibrils in periosteum and bone

5 The ultrastructure of areas of non-fibrillar structure in bony tissue

The cells were best evaluated in H and E and in PAS-sections There were no differences in number and distribution of the cells in normal and otosclerotic stapes. In some preparations the lacunae were found to be empty due to autolysis. This occurred more often in the normal stapes. The collagen fibrils stained best with the van Gieson–Hansen technique and with Sirius-red.

In most preparations the fibrils pass in wavy parallel bundles, mostly along the longitudinal axis of the crus. In a few preparations they take a more irregular coiled course, but this is seen in normal as well as in otosclerotic stapes. In areas of non-fibrillar structure there are no differences in colour distribution or intensity

The elastic fibrils are clearly recognized with silver impregnation Most fibrils are seen in the periosteum and in the perivascular tissue. No difference is apparent in the distribution and the density of these fibrils in normal and otosclerotic stapes. Since their structure is characteristic of normal enchondral bony tissue, they have not been described separately The surface of the crura is covered with periosteum,

in which there are numerous interdigitating fibroblasts, which frequently have irregular nuclei with evenly distributed chromatin and a distinct nucleolus. Their cytoplasm sends out long processess there are numerous delicate fibrils, occasional mitochondria, abundant, rather coarse endoplasmatic reticulum, poorly developed Golgi network. Between the fibroblasts are *bundles of collagen fibrils running* in all directions. Some of the fibrils seem to continue directly into the fibrillar structure of the underlying bony tissue The periodicity is that characteristic for collagen fibrils, approximately 650 A. In numerous areas there are also bundles of extremely delicate fibrils without periodicity presumably representing elastic fibrils The surface of the periosteum is covered by a single layer of densely packed cells, which are distinctly demarcated from each other and from the double-contoured underlying periosteum, though leaving a free passage between the cells of the periosteal stroma and the surface On the free surface of these *cells occasional microvilli are discernible*. The nuclei are regular oval, with densely packed chromatin. The cytoplasm contains numerous mitochondria, occasional vesicles, and abundant granular endoplasmatic reticulum

Adjacent to the periosteum the bony tissue is markedly irregular mostly of a compact structure, which is also seen around canaliculi osteocytes, lacunae and, *very occasionally* between the fibrillar bundles. Both in normal and in otosclerotic stapes there are numerous osteocytes of highly varying size and shape but there are no definite differences between the cells of either origin. In the lacunae there are either cell remnants or more amorphous material a few are emtpy

In the fully decalcified sections collagenic fibrils are seen running mostly in large bundles in various planes. There are however throughout the section, smaller bundles and single fibrils, which run more irregularly Some concentration of fibrils is seen around the osteocytes and the lacunae. The periodicity measured at about 650 A, is constant throughout. The thickness of the fibrils is fairly uniform for one fibril as well as for the fibrils as a whole The distance between fibrils varies greatly

Osteoblasts and osteoclasts have not been observed in any preparation.

DISCUSSION

Since a comparison of normal and pathological tissues in the stapedial footplate and capitulum is rather difficult because of their complicated and specific structure the present study has been confined to the histology of the crura.

The findings are in line with the investigations previously quoted as far as the structure of th. stapedial crura is concerned We failed to demonstrate degenerative changes of the fibrillary structures, as found by Bentzen & Stadil, either in the periosteum or in bony tissues. The fibrillary structure was demonstrated clearly by the staining methods empolyed, and there was no evidence anywhere of true degenerative changes. The appearance of the osteocytes was normal, and in view of the great variations in the shape and size of these cells in normal bony tissue one could not expect to find characteristic features in our preparations anyway Osteoblasts have not been demonstrated, in particularly they were not found in the border zone between periosteum and bone, as is frequently seen in normal bony tissue

The fibrils as a rule were arranged in *bundles as typical of collagen fibrils, and the* ribbons of the collagen periods are evaluated as being identical in normal and otosclerotic stapes. The changes in collagen periods and the course of fibrils, as demonstrated by Reydon & Smith, were not seen in the present study

CONCLUSION

The present histologic investigation did not confirm the theory of otosclerosis being a generalized disorder of connective tissue, since neither light microscopy nor electron microscopic studies revealed structural differences between normal and otosclerotic human stapes.

Neither was it possible to demonstrate histological differences in ossicles or bony tissue as described in previous papers We did not, in the otosclerotic stapes, observe alterations reminiscent of those seen in osteoporosis imperfecta, i.e. defective mineralization or incomplete differentiation of collagen fibrils

ZUSAMMENFASSUNG

Normale und otosklerotische, humane Stapes-crura wurden licht und elektronmikroskopisch untersucht, um zu bestimmen, ob sich generelle histologische Veränderungen der Stapes ausserhalb des otosklerotischen Fokus befinden, die die Theorie von Otosklerose als einem allgemeinem Bindegewebsleiden bestätigen können. Mittels verschiedener Bindegewebsfärbungen wurden 43 Stapes im Lichtmikroskop untersucht, hiervon 31 mit otosklerotischen Fokus. 8 Stapes wurden im Elektronmikroskop untersucht, hiervon 4 mit Otosklerose. Bei der Untersuchung fanden sich Periost und Knochengewebe von normalem Aufbau ohne entscheidende Unterschiede zwischen normalem und otosklerotischen Stapes. Prä-otosklerotische Strukturen wurden nicht gefunden. Es wurden daher keine Veränderungen nachgewiesen, die dafür sprechen, dass Otosklerose ein generelles Bindegewebsleiden sein sollte.

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PILOCARPINE AS A DIAGNOSTIC AID IN THE SCINTILLOGRAPHY OF SIALOPATHIES

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Abstract. A report is given of the results of scintillography of the salivary glands with various pathological conditions, using ^{131}I and ^{99m}TcO$_4$ as the active substance, and the parasympathicomimeticum, pilocarpine nitrate, as an agent selectively to interfere with the distribution and movements of radioactivity between various tissue/fluid compartments. The results show among other things, that utilization of pilocarpine nitrate is of advantage in the evaluation of both the function and the scintillographic appearance of diseases of the salivary glands. Simultaneously the results strongly suggest that conventional scintillography of the salivary glands, performed alone, did not give sufficient information about the nature and localization of the disease. Instead, the function of the glands has necessarily to be examined as well. Hence, it might be understandable that from the viewpoint of the excretory and concentration capacities of the diseased salivary glands, there must be significant differences even between cases belonging to the same clinical group, depending on the prevailing phase of the pathological process.

The use of scintillographic technique in the diagnosis of various pathologic conditions has become safer and easier since the introduction of new more or less tissue-specific radioisotopes, having relatively short half-lives and low radiation energy. Particularly the introduction of technetium ^{99m} (^{99m}Tc) in the form of pertechnetate as a scintillographic agent represents a significant technical refinement in nuclear [illegible] (e.g. Harper et al., 1964; Blahd, 1965; Börner et al., 1965; Smith, 1965; Kazem et al., 1967; Fletcher et al., 1967; Gates & Work, 1967; Grove & Di Chiro, 1968; Editorial, 1968; Abramson et al., 1969). The advantages manifest themselves partly in the biological, partly in the physical, chemical and toxicological properties of the radioisotope. The ^{99m}Tc is rapidly distributed in the intra- and extracellular compartments. It is most likely not incorporated into the thyroid hormonal synthesis; its concentration in the thyroid is dependent upon the iodine trapping mechanism (Harper et al., 1964; Smith, 1964; 196[illegible]; Blahd, 1965; Kazem et al., 1967). Like iodine, ^{99m}Tc is rather selectively secreted into thyroid and into the salivary and the nasal, oral mucosal glands, as well as the g[illegible] glands (Harper et al., 1964; Börner et al., 1965; Alexander et al., 1966, 1967; Fletcher et al., 1967; Gates & Work, 1967; Kazem et al., 1967; Grove & Di Chiro, 1968; Abramson et al., 1969).

In practice, radioisotopes are generally utilized either to detect the anatomic (topographic) distribution within tissues and organs of the active compound, or as an indicator signifying various physiologic processes in the living environment (e.g. Harper et al., 1964; Ullberg & Ewaldsson, 1964; Alexander et al., 1967).

However, scintillographic techniques in which a pharmacodynamically-acting agent should be used to cause an artificial [illegible] and/or mobilization of the radioisotope [illegible] one tissue/fluid compartment to [illegible] relatively few. A priori it would [illegible]

portant or even decisive to use, as an aid, a substance which selectively interferes in the functions of the target subjected to examination (Setälä et al. 1962, 1963 1964 1965 1967)

Salivary glands and the thyroid belong to a group of organs on the functions of which certain pharmaca have a specific effect. Hence, the utilization of "auxiliary" drugs of this nature in connection with the scintillography of the organs must be advantageous. Previous studies have revealed that the functions of the salivary glands can easily be studied by stimulation or inhibition by a number of parasympaticomimethica (Setälä et al., 1962 1963 1964 1965 1967)

Apart from the significance of various pathologic conditions of the salivary glands —that thus represent neurovegetatively labile organs—the glands are important in the maintenance and regulation of mineral/fluid balance (homeostasis) of the internal environment of the body (Rauch, 1959 Alexander et al 1966)

The salivary glands as well as the excretory glands of the intestinal mucosa, have been utilized as routes for the withdrawal of incorporated radioisotopes. Therefore, the transient mobilization of the radioisotope from the internal storages by stimulating the secretion and excretion of the glands by means of subcutaneous pilocarpine nitrate administration has been performed (Setälä et al., 1962, 1963 1964 1965 1967) In man, the volume of saliva excreted as a result of stimulation by pilocarpine nitrate represents at least 600 to 2 500 g in the course of 24 hours. Numerous noxious—some heavy metals, other inorganic substances, certain viruses—can be conveyed in the saliva.

The present study reports observations from studies on patients, suffering from a variety of sialopathies, to whom pilocarpine nitrate was administered in connection with ^{99m}Tc scintillography Because sialopathies may often be connected with pluriglandular disorders, particularly with those of the thyroid, and because pilocarpine nitrate is known to affect to a larger extent the parotid and the submaxillary glands than the sublingual glands, this study considers chiefly the behaviour of the two former salivary gland pairs and that of the thyroid.

MATERIAL AND METHODS

The patients suffering from various salivary gland diseases came from the Otolaryngologic Clinic, and formed an intentionally unselected group from subsequent patients present at the time of this study The number distribution, age and sex of the patients with their respective clinical diagnoses appear in Table I Complete clinical records with relevant results were at the writers disposal. In addition, each patient was re-examined by us personally

The scintillography of the salivary glands and of the thyroid was always performed twice, with an interval of one week between the first and the second examination. The scintillography was carried out at the same time of day the patient having fasted for at least 12 hours. No medication was permitted either on the day of the examination or the day before.

The scintillographic measures were in principle those as presented by Setälä et al. (1967). The commercial device of this laboratory (Magnascanner Picker X Ray Co U.S.A.) was equipped with 5 × 2 in. crystal and a standard black-and-white photorecorder and multicolor recorder The examination of the patients was performed using collimators with the same characteristics, with either 85 or 265 holes. The line spacing was 0.29 mm, the scan speed 34 cm/min. The information was transferred to a rate meter and recorder (an apparatus modified at this laboratory from FH 421 and 20 models Frieseke & Hoepfner Germany)

Two radioisotopes were used, either 0 1 mCi of ^{131}I as sodium iodine in sterile physiologic saline containing phosphate buffer to make the pH 7 (cases 1 and 2) or 1 0 mCi of ^{99m}Tc

as pertechnetate in the form of $^{99m}TcO_4$ (cases 3 to 16) The latter radioisotope was eluted with 40 ml of physiologic saline as a daily basis from a ^{99}Mo-^{99m}Tc-generator (Harper et al., 1964) The two active substances were administered intravenously into the cubital vein. To increase accretion into, and excretion from, the salivary glands and glands in the nasal and oral mucosa, and the thyroid, 20 mg of pilocarpine nitrate was injected subcutaneously 10 min after administration of the radioisotope, 1 hour 10 min prior to the scintillography This measure resulted in the activation of the salivary and the mucosal glands of nasal and oral mucosa, as well as of the sweat glands The excretory phase was interrupted after an additional 20 min by the intramuscular administration of 0.5 mg of atropine sulfate Before starting scintillography the cutaneous surface, the mouth and throat were carefully cleaned to minimize swallowing of radioisotope-containing saliva and mucosal secretion. The activity in blood, saliva and sweat was determined from samples as presented by Setälä et al. (1967) A second scintillography was made after an interval of 7 days without administration of pilocarpine nitrate

The *scintillographic examinations* were performed twice: first with the patrent lying down in the supine position, and then immediately after with the patient in lateral positions.

Because in carrying out radiation measurements the impulse intensity-registering device was not coupled to the appliance, the darkening of the scintillographic films was measured during the runs to obtain quantitative numerical values. On the basis of previous studies at this laboratory it was proved that by employing this method it is possible to achieve a sufficient degree of precision to perform the measurements for which the test series was designed (Setälä et al. 1967 1968)

Briefly the method was as follows. The *scintillographic film* was evaluated on an illuminated transparent table. The surface brightness was evenly regulated by means of precisely adjusted lamps. On top of the film, and in direct contact with it, a photocell was placed. The size of the active surface of the photocell for the present purpose was approx. 5×10 mm. The photocell was mechanically coupled to the mobile mechanism of a recording pen so that it exactly followed the x-axis directional movements of the pen, but was independent of the y-axis directional movements The photocell s output through the medium of an amplifier directed the pen s y-axis movements. By employing non-linear elements in the back-coupling of the amplifier the basic curve of the amplifier was experimentally made, so that the recording pen s movements corresponded directly to the impulse mtensity which caused darkening of the film. By allowing the pen to travel slowly from one edge to the other in the direction of the x-axis, the photocell correspondingly travelled along the film surface on the illuminated table and the recording pen registered the impulse intensity of the band to be measured. In this way the whole film surface was plotted.

To reveal the precise localization of the activity two known points were marked on the patient's skin, both laterally and facially before scintillography The patient s facial and profile views were photographed in natural size On the basis of the skin marks, each photograph and corresponding scintillograph were superimposed, and re-photographed together

RESULTS

The effect of pilocarpine nitrate administration on the distribution of the radioactivity between different tissue/fluid compartments

The radioisotope ^{131}I was used in two instances (cases 1 and 2) for orientation and general control of the technique To provoke accretion into, and excretion through, the salivary glands, 20 mg of pilocarpine nitrate was injected subcutaneously into the patients (first examination) 10 min after the intravenous injection of the radioisotope, and 1 hour 10 min

Table I *Diagnostic data in 16 patients with various salivary gland disorders*

No of case	Age	Sex	Principal salivary gland(s) impaired	Clinical diagnosis
1	37	♀	Left parotid	Sialolithiasis
2	36	♂	Right submandibular	Subacute sialadenitis
3	44	♀	Both parotids	Mikulicz syndrome
4	26	♂	Left parotid	Sialosis
5	51	♀	Both parotids	Gougerot Houwer Sjögren syndrom
6	42	♂	Left parotid	Sialosis. Postradiation sialopathy
7	59	♀	Both submandibulars	Sialadenitis
8	42	♀	Both parotids	Gougerot Houwer Sjögren syndr
9	45	♀	Both parotids	Mikulicz syndrome
10	68	♀	Both parotids and submandibulars	Gougerot–Houwer–Sjögren syndro
11	24	♂	Both parotids and submandibulars	Sialadenitis
12	48	♂	Left parotid	Mik licz syndrome
13	20	♂	Right submand bular	Sequela of sialolithiasis
14	24	♀	Left submand bular	Sialadenitis
15	36	♂	Right submandibular	Sequela of sialolithiasis
16	23	♀	Left parotid	Sequela of operated cyst
			Right parotid	Sialadenitis

prior to scintillography The blood samples were taken 45 min after the administration of the radioisotope The administration of the parasympathicomimeticum resulted in profuse sweating. In most instances this was already evident after 1 to 3 min. Except for three articular cases (cases 3 8 and 10) a more or ess intense salivation occurred The effect of pilocarpine nitrate was interrupted after a collection time of 20 min. Table I gives the concentrations of the radioactive substance in blood, saliva and sweat. To obtain mutually correlatable activities, the values signifying the respective activities were calculated to correspond to an administration dosage of 1 000 μCi (1 mCi) of ^{99m}Tc

Radioactivity in the blood

After the correction of external radioactivity measurements for isotope decay the results show that in the two instances (cases 1 and 2) in which ^{131}I was administered, the activities in the blood were 4 1 and 2.2 nCi/g respectively The situation was different in cases where $^{99m}TcO_4$ was used as an active substance (cases 3 to 16) The average activity in these cases was 53 nCi/g, the highest 120 nCi/g (case 14) Considering that the total blood quantity of an adult man with a body weight of 70 kg averages 4 500 g, the average total activity in the blood can be calculated as 239 μCi/g. In case 14 it thus corresponds to 540 μCi, i.e. 54% of the total dose administered.

Radioactivity in the saliva

Collection of the saliva was carried out for a period of 20 min, after which the flow was discontinued by intramuscular injection of 0.5 mg atropine sulfate. In the course of the collection period a sample with an average of 67 g of saliva was obtained the largest quantity thus collected was 128 g (case 13) Radioactivity measurements revealed that the concentration of ^{131}I in the saliva was 12 times higher than that prevailing in the blood. In three cases (cases 3 8 and 10) the salivary glands did not respond to the effect of pilocarpine nitrate. In all other cases receiving ^{99m}Tc as an active substance, very high quantities of radioactivity were measured. The highest concentration represented a value of 1 000

Response to s.c. injection of 20 mg of pilocarpine nitrate

adio-otope	Total quantity of saliva excreted in 20 ml (g)	Activity Blood (nCi/g)	Saliva (nCi/g)	Sweat (nCi/g)	Total activity excreted in saliva in 20 min (μCi)
I	94	4.1	47	2.6	4 4
I	100	2.2	24	0.72	2.4
Tc	No excretion	30	Nil	10	Nil
T	100	63	370	31	37
Tc	22	42	570	0	13
Tc	107	21	220	0	24
Tc	86	45	670	21	58
T	No excretion	53	Nil	0	Nil
T	19	75	170	18	3
Tc	No excretion	60	Nil	17	Nil
T	84	45	230	18	19
Tc	0.68	43	1000	11	0.68
T	128	36	220	11	28
T	68	120	580	98	39
Tc	62	82	270	27	17
Tc	34	29	240	9	8

nCi/g (case 12) This activity was 23 times higher than that in the blood The average activity in the saliva was 355 nCi/g. An analysis of the mutual relation of the activities in the blood and saliva reveals, first, that the average ratio of saliva/blood radioactivity was of an order of 7.5 second, that the activity in the saliva was not directly dependent upon that prevailing in the blood. In one instance (case 12) the activity in the saliva was 1 000 nCi/g, while that in the blood was 43 nCi/g, i.e. 23 times higher in the saliva. In another instance (case 9) again, the activity in the saliva amounted to 170 nCi/g and that in the blood 75 nCi/g, i.e. only 2.3 times higher concentration in the former Three instances (cases 3 8 and 10) which did not secrete saliva in any detectable quantity showed blood activities of 30 53 and 60 nCi/g respectively It was calculated that 2.4 to 4 4 μCi, or 2.2 to 4 4 % of the total quantity of ^{131}I administered was excreted in the course of the test period of 20 min. Related radioactivity measurements with ^{99m}TcO$_4$ revealed that the corresponding values were 0 68 to 58 μCi, signifying 0 1 to 5 8 % of the total dose injected.

Radioactivity in the sweat

The collection time for the excreted sweat was similarly 20 min, during which sweat was collected from randomly situated skin surfaces. As the intention was only to determine the activity in the sweat samples, the total quantity of the excretion was not determined. The concentrations of I in the sweat in the two instances where this radioisotope was used (cases 1 and 2) were 2 6 and 0 72 nCi/g. Except for cases 2, 5 6 and 8 the average activity in sweat represented a value of 23 nCi/g. In cases 3 and 10 the activities were 10 and 17 nCi/g In three instances (cases 5 6 and 8) the activity in the sweat was nil. In case 8 there were no detectable activities in sweat and saliva, while in cases 5 and 6 no activity was measured in the sweat though high activities appeared in the saliva (570 and 220 nCi/g). As reviewed by Setälä (1965) administration of pilocarpine nitrate into a 70 kg man may result in a total excretion of 1 500 g of sweat; it could be calculated that about 35 μCi would be excretable, i e about 3.5 % of the dose of ^{99m}Tc administered

Table II Distribution of radioactivity with and without administration of 20 mg of pilocarpine nitrate

No. of case	Right parotid P−	Right parotid P+	Left parotid P−	Left parotid P+	Right submand. P−	Right submand. P+
1	+D	++L	+D	++L	+D	D
	+D	++D	+D	++D	+D	++ L
3	+++L	++++L	++D	+++L	++D	+++D
4	+−+L	+++L	+++L	++L	++D	+++D
5	+++D	++++L	++D	+++L	+D	++D
6	+++L	++++L	++L	+++L	++D	+++L
7	+++L	+++L	+++L	+++L	+++L	+++L
8	++D	+D	++D	+D	++D	+ D
9	++L	−++L	++D	+++L	++D	+ +D
10	+D	++L	+D	+++L	+L	L
11	D	+D	+D	+D	+D	D
1	++++L	++++L	++++L	++++L	++++L	++++L
13	−++L	++L	+++L	++L	+++L	+++ L
14	+++L	++L	+++L	++L	+++L	+++L
15	+++L	++L	+++L	++L	+++L	+++L
16	−+D	+D	+D	+D	+++L	+ L

symbols L = local distribution of activity D = diffuse distribution of activity + + + + = very strong, + + + = strong, + + = medium, + = weak.

The effect of pilocarpine nitrate administration on the accretion of the radioactivity to and departure from the salivary glands

The patient data with relevant clinical remarks appear in Table I. Table II demonstrates the localization and concentration of the radioac tivity in the parotid and submandibular glands and in the thyroid, nose and mouth, before d after subcutaneous administration of 20 g of pilocarpine nitrate Depending on the ery extensive numerical material resulting from the measurements, and in order to give an over all view of the effect of the accretion-changing drug, the primary numerical values obtained by the automatic photocell measure ments of the respective scintillographic films were translated into symbols + ++ +++ and ++++ signifying "weak" "medium" "strong" and very strong" activities.

The following 3 case histories with their re sults are presented first.

Case 3

The patient was a female of 44 During the past 5 years she had been suffering from re current swelling and pain sensation within a region corresponding to the parotid glands and with occasionally raised body temperature. In addition, there was a feeling of dryness of the mouth. Examination at hospital revealed that the right parotid gland was enlarged, while the left was not palpable The oral mucosa was of normal appearance Sialoradiography showed diffusely-distributed punctate dilatation of the peripheral salivary ducts bilaterally Needle biopsy from the right parotid gland revealed the presence of cellular elements of the epitheloid and giant cell types, partly arranged in a granulomatous manner (Fig. 4) Diagnosis: Mikulicz syndrome

Scintillography showed a high-degree accretion of the activity in the thyroid as well as in the mucous membranes of the nasal and oral cavities (Figs. 1 2) Administration of pilocarpine nitrate resulted in extensive changes in the topographic distribution. a strong increase in the accretion of the activity in the diseased right parotid gland, as well as in both the submandibular glands, and in the nasal mucosa (Fig. 3) The concentration of the radioactivity in the thyroid remained unchanged.

Interpretation

Intravenous administration of ^{99m}Tc to a patient with Mikulicz syndrome resulted in a

eft submand.		Nose		Mouth		Thyroid	
–	P	P–	P+	P–	P	P–	P
D	+D	+D	++L	D	++L	++ +L	+ + L
D	++D	+D	++D	+D	++D	− +L	− − + +L
+ D	+++ D	++L	++ +L	++D	+++L	+++ L	+ − −L
++D	+++D	++L	+ +L	++++L	+ ++L	++D	++ L
D	++D	− − +L	+L	− +L	++L	− −D	+++L
+ L	+++L	+++ L	+ +L	++++L	+++L	+++L	+L
+L	+++L	++++L	+ L	++ +L	++L	+++L	++L
+ D	++D	+++D	++D	++D	+D	+− L	++ +L
+D	++D	++D	+D	+D	++D	++D	++L
+D	L	++D	+ L	+D	++D	++D	+ L
+D	D	+ D	+D	+ L	+++L	+++L	−− L
++L	+− L	++D	+ D	+D	++D	− +L	+ L
++L	++L	+++ L	+L	+++ L	+ L	−+ L	+ L
+ +L	++L	++ +L	++L	++ L	++L	− +L	− L
++L	−L	++++L	+L	−++L	++L	−− +L	+ L
+D	D	+++D	+D	+−+L	++L	−+ L	− −+L

strong accretion of the activity in the diseased right parotid gland. A prior subcutaneous injection of pilocarpine nitrate caused extensive changes in both the concentration and localization of radioactivity: in particular an increase in the diseased right gland. At the same time the salivary glands did not respond to the excretory effect of the drug and consequently no sample of the saliva was obtained.

Case 6

The patient was a male of 42. For 9 years he had been suffering from recurrent swelling of the left parotid gland. In order to produce an artificial atrophy of the gland, local irradiation (a total dose of 600 R) was given. As a result the swelling disappeared, particularly after ligation of the left Stensen's duct.

Examination revealed clinically normal sali-





Fig. 1. Mikulicz syndrome (case 3). Scintillograms with ^{99m}Tc; right (*a*) and (*b*) left lateral views. Situation without pilocarpine nitrate administration.



Fig 2 Same patient as in Fig. 1 Scintillogram with ^{99m}Tc' anterior view Situation without pilocarpine nitrate administration.

glands. Diagnosis. Sialosis. Postirradiation sialopathy

Scintillography (Fig. 5) showed that the highest radioactivity was located in the nasal and the oral mucosa, and in the thyroid. The left (diseased) parotid gland accreted a lower amount of activity Administration of pilocarpine nitrate resulted in significant changes in the distribution of the activity the concentration in the left parotid gland was increased —though in a distinctly lower degree than that in the right (Fig. 6) The concentration in the thyroid was decreased.

Interpretation

Intravenous administration of ^{99m}Tc to a patient with postirradiation sialopathy of the left parotid gland resulted in a distinctly lower accretion of the radioactivity in the diseased gland. A prior subcutaneous injection of pilocarpine nitrate provoked development of distinct changes in the topographic distribution of the active material, as well as an increase in the accretion into the diseased gland. Concentration of the activity in other salivary glands increased. The concentration in the saliva was extremely high (220 nCi/g), but in the sweat nil.

Case 10

The patient was a female of 68 During the six years before admission to the hospital, she had been suffering from dryness of the mouth, and swollen parotid and submandibular glands. She had carried a bottle of water in her bag to get temporary relief from the sensation of dryness.

Examination revealed swollen parotid and



Fig 3 Same patient as in Figs. 1 and 2. Scintillogram with ^{99m}Tc; anterior view Situation after pilocarpine nitrate administration. Significant drug-provoked alterations in topographic location and concentration of radioactivity (cf. Fig. 2).



Fig 4 Same patient as in Figs. 1 to 3 Microphotograph. Needle biopsy from right parotid gland. Granulomatous lesion with cellular elements f epitheloid and giant cell types. Papa stain. Magnification 400

submandibular glands. The oral mucosa was parched and covered by sticky mucus. There were signs indicating the presence of keratoconjunctivitis sicca. Sialoradiography showed marked variations in diameter of the peripheral salivary ducts, and filling defects suggestive of cystic dilatation. Diagnosis. Gougerot Houwer Sjögren syndrome

Scintillography (Fig. 7) revealed that the general uptake of the activity by all salivary glands was of a low order The secretion in the thyroid was of medium degree. Administration of pilocarpine nitrate increased the degree of secretion only weakly but caused a sharpening of the relief boundaries of the active material, i.e. increased the concentration (Fig. 8)

Interpretation

Intravenous administration of ^{99m}Tc to a patient suffering from Gougerot Houwer Sjögren syndrome resulted in a comparatively low-degree secretion of the radioactivity in all salivary glands, and perhaps in the thyroid as well. A prior subcutaneous injection of pilocarpine nitrate provoked a slight increase in the activity within the glands above, but did not result in any measurable excretion of saliva.

The other cases of the material not reviewed above could be analyzed from several viewpoints. The present authors believe that results from three groups of the material deserve particular attention

The first group of these patients comprises three cases (cases 5 6 and 8) with no measurable excretion of the sweat after administration of pilocarpine nitrate. The radioactivity in the saliva in two instances was 570 (case 5) and 220 pCi/g (case 6) whereas no saliva-



Fig 5 Postirradiation sialopathy (case 6). Scintillogram with ^{99m}Tc: anterior view Situation without pilocarpine nitrate administration.



Fig 6 Same patient as in Fig. 5 Scintillogram with ^{99m}Tc anterior view Situation after pilocarpine nitrate administration. Significant drug-provoked alterations in topographic location and concentration of radioactivity (cf. Fig. 5).

ion occurred in the third (case 8) An analy of the scintillograms revealed that in the wo cases with a profuse salivation and high activity in it, administration of the parasympathicomimeticum significantly increased the accretion rate of the radioactivity in both parotid glands On the other hand, in case 8 in which there was neither sweat nor saliva excretion despite drug administration, the injection distinctly decreased the concentration of radioactivity in the parotid glands the nature of the change and degree of accretion were similarly bilateral.

The second group is represented by a single patient (case 12) diagnosed to be suffering from Mikulicz syndrome Characteristic features of the results were only 0 68 g of saliva could be collected during the test period of 20 min In spite of the low quantity of saliva the concentration of radioactivity in the saliva was extremely high, representing a value of 1 000 nCi/g. Scintillography revealed a very strong accretion of the radioactivity both in the parotid and the submandibular glands bilaterally in a symmetric manner The degree of accretion was of the same order before and after administration of pilocarpine nitrate

The third group of patients subjected to detailed analysis comprises four cases (cases 2, 4 6 as well as case 13) A feature common to all these cases was the high total quantity of the excreted saliva, viz. 100 100 107 and 128 g respectively The clinical diagnoses were: subacute sialadenitis (case 2) with ^{131}I as the active substance and sialosis (case 4) sialosis and postradiation sialopathy (case 6) and sequela of sialolithiasis (case 13) with ^{99m}Tc as an active agent. Scintillographic examination showed that the injection of pilocarpine nitrate more or less distinctly increased the accretion



Fig 7 Gougerot Houwer Sjögren syndrome (case 10). Scintillogram with ^{99m}Tc, anterior view Situation without pilocarpine nitrate administration.

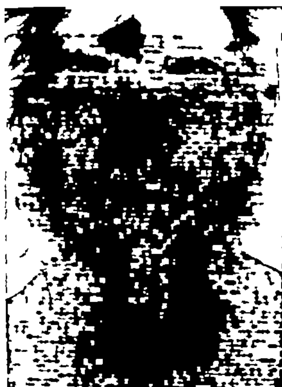


Fig. 8. Same patient as in Fig. 7. Scintillogram with ^{99m}Tc; anterior view. Situation after pilocarpine nitrate administration. Significant drug-provoked alterations in topographic location and concentration of radioactivity (cf. Fig. 7).

of radioactivity in the diseased gland in three instances (cases 2, 6 and 13), while a decrease in the concentration was apparent in one instance (case 4).

DISCUSSION

The present investigation was performed on a group of patients suffering from a variety of salivary gland diseases. The purpose of the study was twofold: first, to demonstrate the advantage of applying a pharmacodynamically affecting drug (the parasympathicomimeticum pilocarpine nitrate) as an aid in the scintillography of diseases of the salivary glands; second, to demonstrate the fact—showed earlier in experimental animals (Setälä et al. 1962, 1963, 1964, 1965)—that the specifically-stimulated saliva excretion can be used as a route for withdrawal of radioactive materials in situations in which a decorporation is indicated. The object of the study was not to examine those salivary gland diseases which cause permanent defects in the filling of the organs with the active substance: the patterns in the latter instances are already known, and the appropriate techniques in every-day use. The chief results of the study were:

1. Utilization of subcutaneous administration of pilocarpine nitrate was advantageous in the context of scintillography of the salivary glands. The drug itself and the dosage employed (20 mg) were not toxic and did not cause any detectable side-effects.

2. The injection of the parasympathicomimeticum into the patients in the context of scintillography resulted in all instances in more or less significant alterations both in the local accretion rate in, and distribution between, the salivary glands and the thyroid. In most cases, the drug administration increased the concentration of the active material either in the parotid or the submandibular glands, or in both gland pairs. In addition there were often alterations in the accretion capacity of the thyroid as well. The changes in the glands were accompanied by an increased excretory capacity of the salivary and sweat glands. Considering the cases of the material which responded positively to the administration of pilocarpine nitrate, the following conclusion may be justified. The mean radioactivity in the saliva was 332 nCi/g and in the sweat 26 nCi/g during the collection time of 20 min. Because a single injection of the drug can result in an excretion of 600 to 2 500 g of saliva and 1 500 g of sweat (reviewed e.g. by Rauch, 1959, and Setälä, 1965) and because excretion and secretion of these glands represent an active cell function, the present observations signify the fact that in most optimal instances almost all radioactivity administered could be decorporated by this technique.

3. However, the prevailing healthy condition of the salivary glands is decisive both in evaluation of the removal of the radioactivity

and in detecting and determining the disease in the glands. In this respect, the following six cases of the material were of particular interest and may be reviewed viz. cases 3 5 8 9 10 and 12. The respective clinical diagnoses were Mikulicz syndrome in three instances (cases 3 9 and 12) and Gougerot Houwer Sjögren syndrome in three (cases 5 8 and 10) As is evident from Tables I and II, features common to the cases were first, the very low or nil excretion of saliva after pilocarpine nitrate administration and second, the observation that a low degree drug-dependent salivation was not as such directly correlated to the secretory capacity of the parotid and submandibular glands. Thus, after specific stimulation by the parasympathicomimeticum, (*a*) the degree (quantity) of the saliva excretion (*b*) the concentration (quantity) of the radioactive substance in the excreted saliva, and (*c*) the changes in the topographic distribution of radioactivity as seen in the scintillography are, in themselves, for natural reasons not directly correlatable However if the three determinations (*a*) (*b*) and (*c*) are carried out side-by-side, much more information can be obtained about the nature of the salivary gland diseases It should also be considered that the basic lesion may often have multiglandular manifestations, and there are several types or developmental degrees of diseases belonging e.g. to the group of Mikulicz and Gougerot Houwer Sjögren syndromes.

It can be emphasized with good reason that in the evaluation of the scintillograms, the morphogenetic and the functional aspects must both be considered. For instance, an observation that a given organ seemingly did not accrete the radioactivity is not sufficient for diagnosis without a parallel control of the function by means of a selectively-affecting auxiliary drug.

ZUSAMMENFASSUNG

Der Bericht betrifft Resultate der Speicheldrüsenszintillographie bei verschiedenen pathologischen Zuständen, u. zw unter Heranziehung von ^{131}I und ^{99m}TcO als Aktivsubstanzen und dem Parasympathicomimeticum Pilocarpinnitrat als Selektivagens zur Einwirkung bei Distribution und Bewegung von Radioaktivität einzelner Gewebe/Liquid-Räume. Wie das Resultat u. a. zeigte, ist die Verwendung von Pilocarpinnitrat auswertungsgünstig sowohl im Hinblick auf die Funktion wie für das szintillographische Erscheinungsbild bei Erkrankungen der Speicheldrüsen. Wie die Resultate ausserdem klar erkennen lassen, gibt eine ohne Zusatzmittel vorgenommene konventionelle Szintillographie der Speicheldrüsen über die Natur und zur Lokalisation der betr. Erkrankung keine ausreichende Information, die Drüsenfunktionen müssen dazu untersucht werden. Hinsichtlich sowohl der Menge wie des Konzentrationsgrades bei der Exkretion erkrankter Speicheldrüsen dürfte es indessen verständlich sein, dass je nach der Phase des pathologischen Prozesses zwischen Fällen derselben klinischen Gruppe signifikante Unterschiede auftreten können.

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θ_H = angular velocity of head
T_1 ≃ Polar moment of inertia of endolymph (J)—viscous torque per unit relative angular velocity of fluid flow (B)
T ≃ B—Moment of cupular restoration force per unit angle of fluid displacement in the canal (K).
k = $\alpha J/K$, where α is the proportionality constant relating cupular angle to angle of fluid displacement.

In this experiment T is the time constant of approach to steady endolymph flow on sudden application of a steady angular acceleration, and as indicated in the above reference is too short to be significant during the patterns of movement employed in these experiments. Equation (1) may therefore be simplified for the present purposes to

$$\frac{\theta}{\dot{\theta}_H}(s) = \frac{ks}{T_c s+1}$$

$$\text{or } \theta\ (s) = \frac{ks\dot{\theta}_H}{T\ s+1} \qquad (2)$$

in which T is the time constant of exponential cupular return due to the interaction of elastic and viscous forces after sudden change in stimulus angular velocity. With this system a step change in angular velocity input will lead to an initial response (cupular deflection) followed by an exponential return to zero response with a time constant T ≃ 16 sec estimated by cupulometric nystagmography (Groen, 1960 Melvill Jones et al., 1964). The form of this basic response is illustrated in Fig. 1 *A*. If the oculomotor response were proportional to the vestibular input, as suggested by results of Hallpike & Hood (1952), this curve should also represent the time course of the slow phase eye angular velocity during nystagmus. However Fig. 3 exemplifies the time course of the slow phase eye velocity actually observed in a human subject during the present experiments. The primary response did not simply decay to zero as would be expected from equation (2) it reversed after 34 sec to yield a prolonged period of secondary nystagmus.

It is suggested in this paper that this form of secondary nystagmus is the result of a superimposed adaptive process. This hypothesis rests on two assumptions. First, signals generated in the canals, proportional to the deflection of the cupula, are compared to a shifting reference level central to the mechanical components of the canal. This reference level, R, changes such that it always tends to minimize the difference between θ and R. Fig. 1 *B* illustrates this point. R continually drifts towards the instantaneous value of the canal signal θ_c, attempting to minimize $\theta - R$. In particular the rate of change of R is assumed to be proportional to the value of the difference $(\theta - R)$

$$\text{Hence } \frac{dR}{dt} = b(\theta - R) \qquad (3)$$

where b is the constant of proportionality. In Laplace notation

$$R(s) = \frac{b\theta}{s+b} = \frac{\theta}{T\ s+1} \qquad (4)$$

where $T = 1/b$ and is the adaptive time constant.

Substituting for θ from equation (2)

$$R(s) = \frac{ks\dot{\theta}_H}{(T\ s+1)(T\ s+1)} \qquad (5)$$

The second assumption is that the slow phase angular velocity of resulting nystagmus is proportional to $(\theta - R)$. The dotted vertical lines in Fig. 1 *B* indicate $(\theta - R)$ while Fig. 1 *C* shows $(\theta - R)$ as a function of time. If θ_{eye} represents the slow phase eye angular velocity relative to the skull during nystagmus, then

$$\dot{\theta}_{eye} \propto (\theta - mR) \qquad (6)$$

The parameter m is included since there is no *a priori* reason why θ and R are viewed with the same gains. If m is not unity but varies according to the direction of rotation, the model behaves as though it had a directional preponderance.

Substituting from equations (2) and (5) for θ and R, the vestibularly driven eye angular velocity (θ_{eye}) becomes,

$$\theta_{eye} = \varrho \left\{ \frac{ks\theta_H}{T\ s+1} - \frac{mks\theta_H}{(T\ s+1)(T\ s+1)} \right\} \qquad (7)$$

where ϱ is the constant of proportionality

This transfer function formally describes the relation between *head* angular velocity as input (stimulus) and resulting slow phase eye angular velocity as output (response) and defines the variables concerned in a manner permitting experimental verification of the hypothesis from which it is derived.

It may be noted here that in practice none of the subjects tested exhibited a significant directional preponderance (response uniformly biased in one direction) and accordingly the parameter m was held at unity throughout

EXPERIMENTAL METHODS

After a number of preliminary experiments to determine suitable stimulus profiles, eight human subjects (3 male, 5 female) ranging from age 18 to 39 and free from overt vestibular or oculomotor pathology were exposed to two sets of stimuli.

The first was a ramp velocity generated by angular acceleration lasting 120 sec and having an amplitude of 4.5 /sec^2 This stimulus was chosen since after approximately 60 sec the cupula should have reached a constant angle of deflection, and any changes in the response after this time should be attributable to adaptation The second stimulus was a step change in angular velocity of 270 /sec requiring 10 sec for completion. This stimulus was large enough to produce a clear secondary response without generating maximum eye velocities so high as to be limited by eye dynamics. In practice, the ramp velocity was achieved by first taking the subject slowly to the appropriate angular velocity in one direction and leaving him in this steady state for 3 min to permit complete cupular restoration. For the ramp velocity profiles, the table was driven to follow the required acceleration through zero velocity to an angular velocity in the opposite direction equal to that of the initial steady condition This procedure was adopted to minimise the maximum *absolute* angular velocity attained by the turntable. The step change in velocity was achieved by taking the subject from a constant velocity to zero velocity

The subjects were rotated while sitting on a servocontrolled rotating chair with their heads fixed to the chair by a dental bite. Their arousal was maintained by having them compete for a monetary reward by working out factorial 10 mentally The instantaneous eye position was recorded by means of D C. electro-oculography (EOG) and static calibration was done at 10° and 20 left and right before and after each experiment

Before experimental runs, all subjects were dark adapted for at least 50 min in red light previously shown to yield EOG gains indistinguishable from complete darkness after this time (Gonshor & Malcolm, in preparation) All calibrations were performed in red light and all experiments in total darkness. The eyes-closed condition was adopted on account of difficulties with lacrymation, blinking and extraneous facial emg activity introduced with eyes open during these high level stimuli.

From the resulting nystagmographic records, beat-by-beat slow phase angular velocity was plotted (*ordinate*) against time elapsed after commencing the rotational stimulus (*abscissa*) as in Figs. 3 and 4 Early results were laboriously measured by hand. But the majority of original records were analysed by means of a tangent analyser similar in principal to that described by Benson & Stuart (1967), but generating a direct write-out in graphical form on an X–Y plotter as in Figs. 3 and 4 The accuracy of eye angular velocity measurement was of the same order of magnitude as the size of the dots in these figures.

In order to match the results obtained in this way with the mathematical model, the transfer function defined in equation (7) was programmed onto an EAI TR 20 analogue

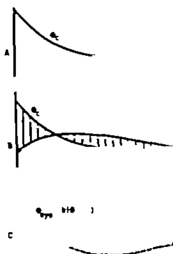


Fig. 1 Form of time dependence of response to step change in head angular velocity. (*A*) Cupular deflection (θ) from resting position. (*B*) The shifting reference level (*R*) which tends to minimize $\theta - R$ (dashed lines). (*C*) Slow phase eye angular velocity ($\dot{\theta}_{eye}$).

computer whose output was displayed on an oscilloscope. The computer could be made to run at ×500 real time, causing the response to any chosen input waveform to appear on the oscilloscope as a complete and continuous curve. The values of the parameters in the equation could be manually adjusted, producing an immediate change in the output curve viewed on the oscilloscope.

The graphs of the eye velocity versus time (such as shown in Figs. 3 and 4) were photographed, and projected by means of an ordinary 35 mm slide projector onto the face of the oscilloscope. The computer was adjusted so that the output time base corresponded to the time divisions of the graph, and the predicted curve was then matched by eye with the observed responses of the subjects. The superposition thus obtained was photographed for subsequent reference through semi-silvered mirrors, the results appearing as in Figs. 5 and 7.

The matching procedure leading to the values of T and T in Table I, used the following criteria for obtaining the best fit between the model and the observations.

(*a*) The parameters T and T were adjusted so that the model should fit with similar accuracy the data from both stimuli for a given subject.

(*b*) Parameters T and T obtained from the visual fit of a given set of data should be reasonably reproducible (see Table I).

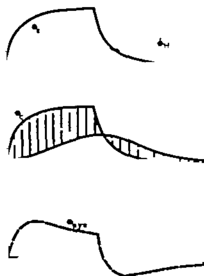


Fig. 2 Form of time dependence of response to ramp velocity of the head ($\dot{\theta}$). (*A*), (*B*) and (*C*) as in Fig. 1.

RESULTS

Figs. 1 and 2 illustrate the forms of response to be expected from the model defined in equation (7) for a condition approximating $T = 4T_e$. In all curves (thick lines) the ordinate indicates response and the abscissa time elapsed after commencing the rotational stimulus. The thin line in Fig. 2 *A* gives the imposed stimulus angular velocity which was a ramp followed by steady angular velocity.

The curves (*A*) in Figs. 1 and 2 represent the change of cupular angle (θ_c) with respect to time. As previously mentioned in the theoretical considerations a stepwise stimulus generates an initial response followed by an exponential decay. The response to a ramp stimulus rises with the same exponential time course as in Fig. 1 *A* to achieve an asymptotic level which is held steady until cessation of the accelera-

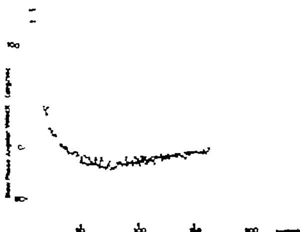


Fig 3 The response of one subject to a sudden change in angular velocity The slow phase angular velocity of the nystagmus is plotted beat by beat against the time after the onset of the stimulus. The eye movement after reversal of direction at 34 sec is secondary nystagmus.

tion (Fig. 2 *A*) On assuming steady angular velocity of stimulus (θ_H) the response decays to zero

In curves (*B*) the hypothetical reference levels (*R*) are *shown with their time course* defined by equations (3) and (4) The vertical dashed lines between θ and R give the value $(\theta - R)$ which determines the instantaneous slope of the reference curve (*R*)

In curves (*C*) the final oculomotor responses, ... as the slow phase angular velocity of ...ting ocular nystagmus and defined by ...quation (7) are plotted. The deviations of curves (*C*) from curves (*A*) are easily seen. Not only is considerable secondary nystagmus evident, but the whole shape of the response is changed in a systematic way It is particularly important to appreciate that if curve 1(*C*) is plotted on log-linear graph paper only the portion above zero is normally visible and gives the impression of a decay which is considerably more rapid than the basic exponential decline in Fig. 1 *A* This matter will be referred to again in the discussion.

Fig. 3 illustrates the plot of resulting slow phase eye angular velocity (*Ordinate*) against time, obtained from one subject by the analytical process described above. Each spot gives the velocity during one nystagmic beat. All nystagmic beats from one experimental run are included. In practice the step change of angular velocity occupied approximately 10 sec, which accounts for the initial rising response The subsequent primary response decayed smoothly through zero into a prolonged secondary phase of reversed nystagmus The values of points on the zero ordinate were obtained from clearly defined horizontal lines on the original eye movement record, interspersed with well-marked *saccades* and could be easily measured. It is incidentally noteworthy that the curve passes smoothly rather than discontinuously, through this zero, the theoretical implications of which will be discussed below

Fig. 4 is a similar plot obtained from the same subject as in Fig. 3 exposed to the stimulus depicted in Fig. 2 *A* and described numerically under experimental methods This was the only record in which "lumping" of data points tended to occur on the zero ordinate. The similarities between the plots in Figs. 3 and 1 *C*, and 4 and 2 *C* are striking and form the basis of the numerical analysis

Figs 5 *A* *B* are photographs of the actual fits obtained on the scope face as described under Methods, for the two plots shown in Figs 3 and 4 respectively

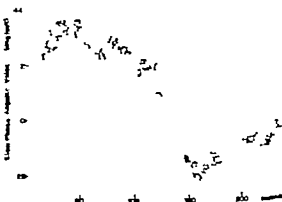


Fig 4 The response of one subject to a velocity ramp. The slow phase angular velocity of the nystagmus is plotted as a function of the time after onset of the stimulus. The arrow indicates the time after which the angular velocity of the head was held constant.

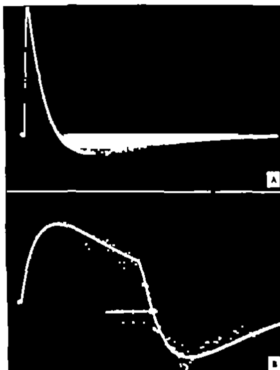


Fig. 5 The photographs show the superposition f the experimental data ( ), and the output of the analogue computer (——). The upper response is from sudden change in angular velocity while the lower one is from velocity ramp. The *ordinates* give slow phase angular velocity of the ystagmus, while the abscissae represent the time after the onset of the stimuli.

The values of the time constant of cupular restoration (T) and the adaptive time constant (T_a) calculated from the relevant potentiometer settings on the analogue model of equation (7), are given in Table I for all subjects and all experiments. The two columns under each parameter heading give duplicate values obtained from curve fittings on a single set of data plots performed with 10 days interval between the fitting procedures, a period sufficiently long to forget prior knowledge of results. The duplicate sets of results for each time constant indicate reasonable reproducibility

The standard deviation and standard error values are calculated from the combined re sults from first and second fittings. Mean values for cupular time constant and adaptive time constant were $T = 21$ sec (S.E. 1.5) and $T =$ 82 sec (S.E. 6.5) respectively

Table I

	T (sec)		T (sec)	
Subject	*A*	*B*	*A*	*B*
JR	17.5	14	63	66
DC	15.5	15[illegible]	82	81
VS	18	[illegible]3	105	93.5
JO	[illegible]3	20.5	66	66
CN	[illegible]1	20.5	125	150
LS	26	29.5	78	64
DP	30	3	58.5	53
AF	15.3	14	81	83
Mean	21		8	
S.D.	5.9		25.8	
S.E.	1.5		6.5	

Experimental values for the canal cupular restoration time constant (T_c) and the adaptation time constant (T) for each of the subjects tested. Columns *B* are alues obtained 10 days after those in column *A* Mean, S D and S.E. values are calculated from the combined data in columns *A* & *B*.

DISCUSSION

Results such as those exemplified in Figs. 3 and 4 demonstrate dramatically how wide the divergence of physiological response to rotational stimulation of the canals can be from that predicted by the simple torsion pendulum model usually considered a fair approximation of the cupular-canal-endolymph system The fact that the recorded divergence of these objective re sults could in all subjects and all experiments be adequately accounted for by the adaptive model here proposed, strongly suggests that such an adaptive function, or one closely resembling it, is in fact constantly active in all circumstances. It appears that a similar phenomenon may account for subjective effects as well (Young & Oman, personal communication)

The findings raise the question, what func tional role could be served by the adaptive ef fect here described? In attempting to answer this question it is important to appreciate first that the long time constant attaching to the adaptive phenomenon appropriately precludes it from interfering significantly with the active vestibular sensory message during the relatively short, sharp head movements of everyday life On the other hand, long time constant adapta-

tion would be highly effective in tending to maintain, over long periods, the steady state, or D C., balance of the differential inputs impinging on the central nervous system (CNS) from the two sides of the head. The physiological implications of this become apparent when it is appreciated that the average neural discharge from each ampulla has a resting value which is increased by rotation in one direction and decreased by rotation in the other direction (Löwenstein & Sand, 1940 Adrian, 1943 Gernandt, 1949 Groen 1952) As pointed out by Melvill Jones 1965 *a* this evidence coupled with results of unilateral canal plugging experiments (Money & Scott, 1962 Zuckerman, 1967) indicates that the CNS acts differentially upon the signals from pairs of canals. Let the resting discharge rates from a pair of opposite canals be A and B respectively It may then be postulated that reflex response to canal stimulation will be in proportion to the differential term $A-B$ which, for D C. balance, may or may not be zero in the stationary condition. But during skull rotation, since each canal is a mirror image of its contralateral counterpart, the change in firing rates will be from A to $(A+\Delta A)$ and B to $(B-\Delta B)$ The CNS would then "see" these two inputs differentially and compare the new re t with the resting condition (i.e $(A+\Delta A-+\Delta B)$ to $(A-B)$) In this notation the relevant change amounts to $\Delta A+\Delta B$ But presumably such a change would be indistinguishable to the CNS from a change in the value $A-B$ due to natural biological drift or some pathological cause However if the reference level R proposed above always shifted towards the difference $A-B$ an effective D C balance would be maintained indefinitely The process could be akin to automatic maintenance of the D C. balance in a differential amplifier The value of such a feature in the canal vestibular system is further emphasized by the fact that the sensory signal is essentially one of angular velocity and hence a maintained signal, even though very small, would in time indicate a large change in angular position.

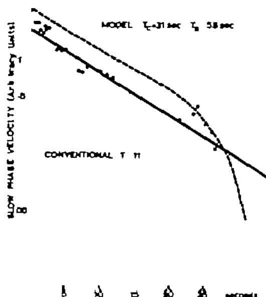


Fig 6. The log of the slow phase angular velocity of nystagmus plotted as a function of time from the results in Fig. 7 If adaptation did not occur the points should lie along a straight line, giving mistaken value for T of 11.5 sec from these results. The dotted line shows the computer fit based on T equal to 31 sec. The semi-log scale exaggerates the error between the points and the dotted line in Fig. 7

In the present context, the significance of this latter observation is highlighted by the fact that in curves such as that in Fig. 3 the area under the primary and secondary responses are of similar magnitude. Since the basic plot is here one of angular velocity this implies that the total angle (integral of angular velocity with respect to time) of primary response *is roughly equalled by* the opposite secondary one Since one s sense of attitude in space is determined by the impression of angular displacement at any given time, the rather surprising conclusion may be drawn that the secondary response if sufficiently above "threshold" can exert an influence of the same order of magnitude as the primary one

It is of interest to note the rather long value of 21 sec obtained for the mean value of the cupular restoration time constant (T_c) This value is considerably greater than those quoted

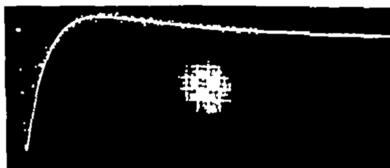


Fig. 7. The entire curve from which the points and dotted curv were taken for Fig. 6. The dots represent the experimental points while the solid line represents the computed response.

under theoretical considerations. The difference can readily be accounted for by the fact that the earlier values were essentially obtained from data points restricted to the primary response largely on account of the fact that results were usually plotted on log-linear graph paper. Since from the torsion pendulum model the response to a step change in stimulus angular velocity should be an exponential decay it has been customary to approximate the plotted response with a straight line, the slope of which then gives the required time constant. Such a plot for one of the present subjects is given as the straight line in Fig. 6. From the slope of this line a value of 11.5 sec emerges for the cupular restoration time constant. But adopting the best fit to the whole set of data, as depicted in Fig. 7 (which represents the same data as in Fig. 6 but with primary response displayed downwards in this case) a value of 31 sec emerges for T for this individual, which is more than double the value estimated in the customary manner. The intermittent line in Fig. 6 represents the fitted line of Fig. 7 superimposed on the log-linear plot of data. It may be noted that the separation of the two lines in Fig. 6 corresponds to the small deviation of points from the upwards sweep of the continuous line in Fig. 7.

This is perhaps an extreme example, but it suffices to indicate an inherent error in assessing definitive parameters of the canal system using the method illustrated in Fig. 6. The present considerations suggest that the tendency in the past has been to underestimate the canal time constant as a result of the adaptive effect here described modifying the end-organ response before generation of the functional physiological one.

Some single-cell recordings from primary vestibular neurones of the ray-fish have shown a similar pattern of behaviour (Groen et al. 1952). Firing frequency after changing in response to a change in angular velocity tended to overshoot the resting frequency and only slowly return. This leads one to speculate as to the possible site of this adaptive process. Possibly the shift in reference level represents a shift in ions which occurs within the hair cells of the crista, so as to compensate the generator potential, following cupula deflection. Perhaps, as has been suggested by Löwenstein (personal communication) it may represent a depletion of the synaptic transmitter of the hair cells. Alternatively the adaptation may manifest as central feed-back to the periphery via the efferent pathways (Gacek, 1960) resulting in a sensitivity or gain change of the transducer. And finally a number of central mechanisms might combine to bring about the effect. It should be emphasized that the mathematical model is incapable of discriminating between such processes, and cannot therefore shed any light on their source. It could be that some or all of the above are acting simultaneously.

As mentioned earlier Fig. 3 shows that the response tends to cross the zero axis with little or no discontinuity. This poses the problem as to whether or not a threshold exists. Fig. 8 *A* shows diagrammatically what one would expect to find if a threshold to cupula deflection exist

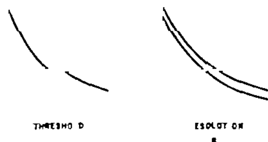


Fig 8 (*A*) The form of the response to a step change in head velocity expected (slow phase angular velocity of nystagmus versus time) if a threshold to cupula deviation existed. (*B*) The form of the response to a step change in head angular velocity to be expected if only finite resolution of cupular deflection existed. The response should tend to lie within the two lines, which represent the resolution limits.

ed During the time when the cupula was passing through its sub-threshold region of deflection, one should get no nystagmus, resulting in a discontinuous curve as shown in Fig. 8 *A* However if the problem was one of resolving the angle of cupula deflection, then the eye velocity during nystagmus would lie between the two lines shown in Fig. 8 *B* The similarity between Fig. 3 and Fig. 8 *B* leads to the conclusion that resolution of one angular acceleration from another is really the problem, and that if a threshold does exist, it is probably very ll It should be pointed out, however that form of threshold would exist if there was friction between the cupula and the walls of the membranous ampulla. This would only be seen when the subject was rotated from a resting position, and would disappear once he was moved. Should this be the case, the threshold for perception of a change in angular acceleration for a subject who has been at constant angular acceleration should be greater than for a subject who has just previously been exposed to a change in acceleration. This point is of particular importance to pilots, since the thresholds found for humans during controlled experiments on smooth moving platforms may be quite large compared to what the pilot can sense in a constantly moving aircraft.

There are additional implications in the applied context of aviation. First, the unnaturally large and/or prolonged rotational stimuli commonly experienced in flight manoeuvres probably generate at least temporarily residual unidirectional effects in the vestibular system (Caporale & Camarda, 1958) Hence an adaptive capability may represent an important functional asset which is normally active in offsetting such an effect. Possibly failure to do so may be associated with generation of the biased impressions of attitude often referred to as "the leans" On the other hand, such "leans may be due to the secondary effects which this paper attributes to adaptation. The methods here described provide the basis for tests by which adaptive capability might be assessed before selection for flying duties. Second, alteration of adaptive capability by the flight environment may be important in achieving proficiency as a pilot and is now amenable to testing. Third, as inferred in a general context above, the functional significance of the secondary response may in some circumstances be approximately equal to the primary one. Presumably in violent rotational manoeuvres, such as repeated rolls and aerodynamic spinning the adaptive term stands to introduce adverse effects which would not be accounted for by previously described physiological phenomena (Melvill Jones, 1965 *b*). Fourth, it is clearly important to incorporate the adaptive function in any model aimed at permitting calculation of the overall vestibular response to movement (Young, 1969)

From the clinical standpoint the results indicate a certain lability in the conventional cupulometric turning test. First the adaptive mechanism could mask periferal pathology Second, it could be that pathological involvement of the adaptive mechanism might itself prove to comprise a significant clinical entity

Finally it is of interest to speculate on the extent to which the present results reflect biological adaptive functions in other sensory channels. Possibly the adaptive principle and analytical methods here described could be employed to examine this question on a quantitative basis.

ZUSAMMENFASSUNG

Ein mathematisches Schema für kurzfristige Anpassung an vestibuläre Stimulation wurde präsentiert. Es wird eine Versetzungs-Funktion hervorgerufen, welche die Langsam-Stufenwinkel-Geschwindigkeit aus resul*tierendem Nystagmus mit der Kopfrotations-Winkel*geschwindigkeit in Verbindung bringt. Das daraus entstandene Schema wurde geprüft, indem man seine Reaktion auf kontrollierte Stufen- und Steigungs-*inkel-Geschwindigkeits-Stimulation mit denen von* acht menschlichen Versuchspersonen verglich. In allen Fällen hat man eine enge Anpassung erhalten. Die Durchschnittskonstante des anpassungsfähigen Gliedes war 82 Sekunden (*mittlere Irrtumsquote 6.5*) und die cupular Wiederherstellungs-Durchschnittskonstante (*T*) war 1 Sekunden (mittlere Irrtumsquote 1.5).

Es wird darauf hingewiesen, daß die vorherigen für *T* errechneten Werte, Unterschätzungen des wahren Wertes darstellen, hervorgerufen durch die Überstellung des anpassungsfähigen Gliedes, welches hier beschrieben ist. Das anpassungsfähige Glied erklärt gut das Phänomen des zweitgradigen Nystagmus besonders bei entweder stärkerem Stimulus oder lang andauernder Rotation. Einige Verwicklungen der Entdeckungen in Bezug auf klinische und Luftfahrt-Medizin wurden besprochen.

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DIE ABHÄNGIGKEIT DES THERMISCHEN NYSTAGMUS VON TEMPERATURVERÄNDERUNGEN AM HORIZONTALEN BOGENGANG

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(Eingegangen am 16. Januar 1970)

Abstrakt. In 29 Messungen an antrotomierten Patienten werden Abkühlung am Bogengang sowie Nystagmus registriert. 15 Versuche wurden mit 0°C und 14 mit 20°C Wasserspülung durchgeführt. Nach einer Latenzzeit von 10–15 sec nach Spülbeginn sinkt die Temperatur am Bogengang um 0,5 bzw 0,8°C ab nach einer Latenzzeit von 20 bzw 25 sec tritt Nystagmus auf. In Tabellen und graphischen Darstellungen zeigt sich ein analoger Verlauf von Abkühlung und Nystagmus mit einem Maximum nach 1,5 min. Bei der 0°C Spülung ist die Nystagmusreaktion entsprechend der stärkeren Abkühlung intensiver Der Nystagmus erlischt in der Wiedererwärmungsphase bei einer noch bestehenden Temperaturdifferenz zur Ausgangslage von 0,3°C. Bei einer stärkeren Abkühlung am Bogengang kommt es zu einer hnelleren Wiedererwärmung, so daß sich hieraus die annte Tatsache erklärt, daß ein starkerer ther er Reiz eine nur geringe Verlängerung des Nystagmus bedingt.

Durch die mit dem Nobelpreis ausgezeichnete Veröffentlichung von Bárány aus dem Jahre 1906 wurden die Dreh- und thermischen Prüfungen als Untersuchungsmethoden in die Klinik einbezogen. Den auftretenden Nystagmus erklärten Mach (1875) und Breuer (1903) aus der Endolymphströmung im Bogengang. Steinhausen (1936) konnte die Wirkung der Lymphströmung durch Beobachtungen der Cupulaablenkung an lebenden Fischen eindeutig nachweisen Die Ursache der Endolymphbewegung ist eine Temperaturänderung, hervorgerufen durch eine Wasserspülung im Gehörgang. Diese vermutete Temperaturänderung bestätigten Schmaltz & Vögler (1924) sowie eigene Untersuchungen Wir konnten 1969 durch komplizierte Versuchsbedingungen die Registrierung des Nystagmus nicht sicher vornehmen Daher möchten wir in einer neuen Versuchsreihe über die Abhängigkeit der Endolymphbewegung sichtbar am Nystagmus, von den Temperaturveränderungen am horizontalen Bogengang berichten.

METHODIK

Die Untersuchungen nahmen wir an antrotomierten Patienten 4 bis 5 Tage post operationem vor nach Abklingen der entzündlichen Erscheinungen. Es wurde retroaurikulär die eine Lötstelle des Thermoelementes eingeführt und am horizontalen Bogengang fixiert. Mit dem verwendeten Galvanometer betrug die Ablesegenauigkeit 0 05 C. Der thermische Reiz bestand aus 2 ml Wasserspülung mit einer Einwirkungszeit von 20 sec. Das Wasser hatte eine Temperatur von 0 und 20 C. Eine Warmwasserspülung kam infolge einer begrenzten Belastbarkeit der Patienten nicht zur Anwendung.

Das Ablesen der Temperatur am Differentialthermoelement erfolgte alle 10 sec während Frequenz und Stärke des Nystagmus alle 15 sec registriert wurden Die Kopfhaltung der Patienten entsprach der Brüningschen Optimumstellung.

Tabelle I. *Temperaturveränderungen am horizontalen Bogengang und Nystagmus bei 0°C Wasserspülung* (15 Versuche)

Obere Zahlenreihe: Temp Veränderung I Grad C. Untere Zahlenreihe Nystagmusfrequenz pro 15 sec.

Versuch	0	0,5	1	1,5	2	2,5	3	3,5	4	4,5	5	5,5	6	6
1	0	0,1	0,4	0,6	*0,7*	0,7	0,7	0,6	0,6	0,5	0,5	0,4		
	0	6	24	25	*25*	20	15	15	9	9	7	0		
2	0	0	0,3	0,5	*0,6*	0,6	0,5	0,5	0,4	0,4	0,3	0,3	0,3	0,1
	0	5	25	27	*27*	27	19	19	11	10	10	9	6	0
3	0	0,2	0,7	*0,8*	0,7	0,7	0,7	0,6	0,6					
	0	23	25	*26*	26	21	21	9	0					
4	0	0,2	0,6	*0,6*	0,5	0,4	0,4	0,3	0,3					
	0	24	27	*33*	25	24	14	14	0					
5	0	0,6	1,1	*1,3*	1,2	1,1	0,9	0,7	0,5	0,5	0,4	0,4		
	0	20	33	*36*	29	23	19	14	10	7	5	0		
6	0	0,6	0,9	*0,9*	0,8	0,7	0,6	*0,5*	0,4	0,4	0,3	0,3		
	0	17	26	*30*	21	20	20	15	10	9	3	0		
7	0	0,4	1,1	1,7	*1,8*	1,7	1,5	1,3	1,2	1,1	1,0			
	0	4	8	12	*18*	9	6	5	4	2	0			
8	0	0,7	1,2	*1,4*	1,3	1,2	1,1	1,0	0,8	0,7	0,6			
	0	1	11	*15*	9	9	6	3	2	1	0			
9	0	0,4	0,8	*1,0*	0,9	0,7	0,6	0,4	0,4	0,3				
	0	1	9	*12*	8	7	5	2	1	0				
10	0	0,2	0,7	*0,8*	0,7	0,6	0,6	0,5	0,4					
	0	14	20	*27*	19	12	8	6	0					
11	0	0,2	0,7	*0,8*	0,7	0,6	0,6	0,5	0,4					
	0	16	23	*27*	22	10	5	5	0					
12	0	0,1	0,2	*0,3*	0,3	0,3	0,3	0,2	0,2	0,2	0,[illegible]	0,1		
	0	12	25	*26*	20	20	20	20	17	10	5	0		
13	0	0,1	0,2	0,3	*0,3*	0,3	0,3	0,2	0,2	0,[illegible]				
	0	16	16	26	*30*	15	14	12	4	0				
14	0	0,1	0,8	*1,0*	1,0	0,9	0,8	0,7	0,6	0,5				
	0	26	39	*41*	41	37	29	25	13	0				
15	0	0,1	0,2	0,3	*0,3*	0,3	0,2	0,2	0,2	0,2				
	0	24	35	36	*38*	33	25	20	10	0				
Durchschnitt		0,26	0,46	*0,82*	0,79	0,72	0,66	0,55	0,49	0,42	3,9	3,7	3,5	
		14	23	*27*	24	19	14	11	6	7	2	1	0	

ERGEBNISSE

Wir führten insgesamt 29 Messungen durch. 15 bei thermischem Reiz mit 0 C und 14 mit 20 C Wassertemperatur In der Tabelle I sind die Versuche mit Eiswasserspülung wiedergegeben.

In den Spalten der Tabelle I bedeuten die oberen Werte die gemessene Abkühlung in Grad Celsius am Bogengang, die unteren Werte die Schlagzahl des Nystagmus pro 15 sec. Neben der Frequenz des Nystagmus registrierten wir die Stärke des Ausschlages. Der besseren Übersicht wegen wurde dieser Punkt in der Tabelle nicht erfaßt, da die Frequenz sehr anschaulich die Bogengangsreizung widerspiegelt.

Das gleiche gilt für Tabelle II die das Ergebnis der 14 Versuche mit 20 C Wasserspülung aufzeigt.

Über Temperaturmessungen am menschlichen Bogengang haben wir bereits 1969 ausführlich berichtet. Unsere jetzigen Werte decken sich mit den bereits angegebenen. Di

Tabelle II *Temperaturveränderungen am horizontalen Bogengang und Nystagmus bei 20°C Wassertemperatur* (14 Versuche)

Obere Zahlenreihe: Temp.Veränderung in Grad C. Untere Zahlenreihe: Nystagmusfrequenz pro 15 sec.

Versuch	0	0,5	1	1,5	2	2,5	3	3,5	4	4,5	5 min.
1	0	0,4	0,7	0,7	*0,8*	0,8	0,8	0,8			
	0	0	13	*13*	10	10	5	0			
2	0	0	0,1	0,2	*0,3*	0,3	0,2	0,2	0,2		
	0	0	14	14	*15*	8	7	5	0		
3	0	0 1	0,3	*0,3*	0,3	0,3	0,2	0,2			
	0	0	18	*18*	18	13	7	0			
4	0	0,3	*0,8*	0,6	0,6	0,6	0,6	0,6			
	0	21	21	*25*	25	15	10	0			
5	0	0,3	0,7	*0,9*	0,8	0,7	0,7	0,6	0,6	0,5	0,5
	0	12	23	*24*	20	17	16	13	10	5	0
6	0	0,2	0,7	*0,8*	0,7	0,7	0,6	0,5	0,4	0,3	0,3
	0	24	*24*	16	17	17	16	10	10	5	0
7	0	0,1	0,2	*0,2*	0,2	0,2	0,2	0,1	0,1		
	0	4	4	*8*	5	5	5	3	0		
8	0	0,2	0,6	*0,8*	0,8	0,7	0,7	0 6	0,6		
	0	*1*	1	*5*	5	5	4	3	0		
9	0	0,2	0,5	*0,6*	0,6	0,5	0,4	0,4			
	0	10	10	*12*	12	6	3	0			
10	0	0,1	0,3	*0 4*	0,4	0,3	0,3	0,3	0,3		
	0	11	15	*20*	12	10	6	3	0		
11	0	0,2	0,3	*0 4*	0 4	0,4	0,4	0,3	0,3	0,3	
	0	22	24	*35*	25	20	17	9	5	0	
12	0	0,1	*0,2*	0,2	0,2	0,2	0 1				
	0	5	*20*	18	15	7	0				
13	0	0,2	0,4	0,5	*0,5*	0,5	0,4	0,4	0,4		
	0	20	36	38	*39*	30	20	15	0		
14	0	0,2	0,4	*0,6*	0,6	0,5	0,4	0,4	0,3		
	0	15	30	*30*	25	23	15	10	0		
rch-mitt		0,19	0,44	*0,51*	0,51	0,48	0,43	0,40	0,36	0,32	
		10	18	*20*	17	13	10	5	2	0	

kühlung mit 0 C Wassertemperatur betrug im Durchschnitt 0,82 C, mit 20 C Wassertemperatur 0,51 C Als Ergänzung möchten wir hinzufügen, daß die Temperaturwelle im Durchschnitt rund 15 sec nach Beginn der Spülung am Bogengang eintrifft. Nach 10 sec bestehen keine Temperaturveränderungen, nach 20 sec bereits 0,11 C.

Durch die Aufzeichnung des Nystagmus läßt sich darüber hinaus ein Einblick in den Grad der ausgelösten Vestibularisserregung in Abhängigkeit von den Temperaturveränderungen am Bogengang gewinnen. Hier sei besonders auf die Punkte wie Beginn, Maximum und Erlöschen des Nystagmus nach Kaltwasserspülung eingegangen.

Der Nystagmus setzt bei geringen Temperaturveränderungen von durchschnittlich 0,11°C ein. Wir ermittelten in der Gruppe der Eiswasserspülungen einen Wert von 0,12 C und in der Gruppe mit 20 C Wasserspülung einen Wert von 0 11 C. Allerdings fand sich eine Schwankungsbreite von 0–0 7 C. Die Zeiten von Beginn des thermischen Reizes bis zum Einsetzen des Nystagmus betrugen 20 sec (0 C) und 25 sec (20 C)

Entsprechend der zunehmenden Abkühlung am Bogengang verstärkt sich der Nystagmus

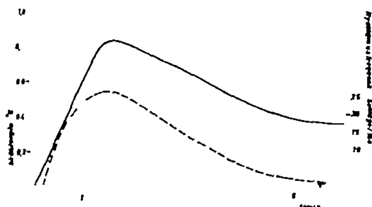


Abb. 1 Abhängigkeit des Nystagmus von der Temperaturveränderung am horizontalen Bogengang nach thermischer Reizung mit Wasser von 0°C. ——— Abkühlung als Funktion der Zeit· ---- Nystagmusfrequenz als Funktion der Zeit.

Dabei erreicht er meistens sein Maximum im Zeitraum der größten Temperaturveränderung. Aus den Tabellen I und II geht hervor, daß nur in 3 Versuchen eine geringfügige Abweichung von dieser Regel besteht, und zwar in der Gruppe mit 20 C Wasserspülung. Die Temperaturveränderung liegt hier bei der größten Nystagmusfrequenz mit 0 03 C niedriger. Bei der Gruppe mit 0 C Wasserspülung decken sich im Maximum der Abkühlung am Bogengang von 0,82 C beide Werte.

Graphische Darstellungen veranschaulichen den parallelen Verlauf von Temperaturveränderungen am Bogengang und die Nystagmusfrequenz.

Nach einer Latenzzeit von 20 sec kommt es zu einem steilen Anstieg der Nystagmusfrequenz bis zum Maximum, das nach 1,5 min erreicht wird. Der langsamere Abfall steht im Verhältnis 1 4 zum Anstieg. Temperatur und Nystagmuskurven nehmen einen annähernd analogen Verlauf. Der Nystagmus erlischt nach 6 min, obwohl der thermische Reiz mit einer noch bestehenden Temperaturdifferenz von 0,35 C nicht kompensiert ist. Für die Kurvenpunkte wurden die Durchschnittswerte der Tabelle I verwendet.

Bei der Spülung mit 20 C Wassertemperatur zeigen die beiden Kurven ein ähnliches Bild.

Die Latenzzeit beträgt _5 sec das Maximum der Nystagmusfrequenz wird nach 1,5 min erreicht. Die Erregung des Vestibularorgans verebbt etwas schneller als der thermische R.

Charakteristisch für beide Kurvenverlauf von 0 und 20 C Wassertemperatur ist, daß der Nystagmus bei einem Temperaturabfall von 0 11 C einsetzt, daß das Maximum der Vestibulariserregung mit der maximalen Abkühlung übereinstimmt und daß der Nystagmus in der Phase des Wiederanstieges der Temperatur am horizontalen Bogengang bei einer noch bestehenden Differenz zur Ausgangslage von rund 0,3 C aufhört.

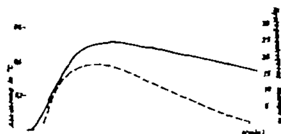


Abb. 2 Abhängigkeit des Nystagmus von der Temperaturveränderung am horizontalen Bogengang nach thermischer Reizung mit Wasser von 20°C. ——— Abkühlung als Funktion der Zeit· Nystagmusfrequenz als Funktion der Zeit.

DISKUSSION

Unsere erneuten Temperaturmessungen am horizontalen Bogengang, die im Kurvenverlauf mit unseren Ergebnissen aus dem Jahre 1969 übereinstimmen, berechtigen uns zu der Annahme, daß die Temperaturverläufe nach thermischer Reizung ein recht reales Bild der

Bárány (1906) niedergelegten Annahme einer Temperaturveränderung am Bogengangssystem vermitteln Die gleichzeitige Nystagmusregistrie rung zeigt die annähernd analoge Reizbeant wortung. Dabei ist die nahezu prompte zeitliche Übereinstimmung beider Kurven deutlich, die auf einen relativ raschen Strömungsbeginn der Endolymphe nach der auftretenden Temperaturwelle schließen läßt. Der Nystagmus setzt lediglich 5–10 sec nach dem Eintreffen der Temperaturwelle ein wie aus dem Zeitpunkt der Temperaturveränderung am Bogengang und dem Nystagmusbeginn deutlich wird. Übrigens deckt sich die Latenzzeit des Nystagmus mit den Angaben von Pfaltz (1958) der eine durchschnittliche Latenzzeit von 19,3 sec bei 30 C und 44 C Wasserspülung ermittelte

Setzt man ein adäquates Verhalten von Endolymphströmung und Nystagmusfrequenz voraus, so kommt proportional der Temperatur veränderung ein schnelles Absinken der Endolymphe im Bogengang mit Zunahme der Strömungsgeschwindigkeit bis zum Maximum der Abkühlung zustande. Bei der einsetzenden Wiedererwärmung verlangsamt sich der Strom, um bei Ausgleich der Temperatur zwischen latera lem und medialem Teil des horizontalen Bogenganges im Sinne der Ausbreitung der Kälte welle nach Jongkees (1953) zum Stillstand zu mmen Der Ausgleich der Temperatur im Bogengangssystem scheint in der Wiederan stiegsphase bei 0,3 C zu liegen.

Eine Umkehrung der Nystagmusrichtung, wie sie von Kobrak (1918) de Kleyn & Versteegh (1924) und Jongkees (1953) beobachtet und von Schmaltz (1932) berechnet wurde, haben wir nicht festgestellt.

Nach der Theorie von Steinhausen (1936) käme mit zunehmender Abkühlung demnach eine Ablenkung der Cupula, mit der Wiederer wärmung und dem Nachlassen der Endolymphströmung eine Rückwärtsverlagerung der Cu pula in Frage.

Für die Tatsache, daß ein stärkerer ther mischer Reiz zwar Frequenz wie Schlagweite des Nystagmus erhöht, jedoch keine wesent liche Verlängerung des Nystagmus hervorruft (Mittermaier 1954) glauben wir aus unseren Messungen eine Erklärung ableiten zu können. Ein stärkerer Kältereiz führt zu einer größeren Abkühlung des Bogenganges (Abb 1 2) und verursacht ohne Zweifel eine schnellere Endolymphbewegung, wie die erhöhte Nystagmusfre quenz beweist. Es kommt bei einer stärkeren Abkühlung zu einer schnelleren Wiedererwär mung und damit zu einer stärkeren Hemmung der Lymphbewegung. Bei der 20 C Spülung erwärmt sich der Knochen nach dem maxima len Abfall in den darauffolgenden 3 min nur um 0,2 C, bei der 0 C Spülung dagegen um 0 4 C. Damit kommt der Eiswasserspülung, entgegen der Meinung von Fitzgerald & Hallpike (1942) eine praktische Bedeutung zu, wenn man Frequenz und Amplitude des Nystagmus berücksichtigt.

SUMMARY

In 29 measurements we recorded refrigeration of semicircular canals and nystagmus by patients with antrotomy 15 experiments were performed by filling the auditory external canal with water of 0 degree Celsius and 14 with 20 degree Celsius. The temperature near the semicircular canal declined for 0 5 or 0,8°C 10 up to 15 sec after filling the auditory canal After latent period of 20 or 25 sec nystagmus appeared. By the tables and graphic representations we can establish an agreement between maxima of refrigeration and nystagmus after 1.5 min. The nystagmus reaction in accordance to refrigeration is most intensive by 0°C water filling. The nystagmus disappeared in the reheating phase by 0.3°C temperature difference relative to starting point. By a most intensive refrigeration of the semicircular canal a quick reheating resulted, so that we can explain the well known fact: a more intense thermal stimulus causes only an insignificant prolongation of the nystagmus.

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FURTHER STUDIES ON THE RELATIONSHIP BETWEEN MENIÈRE, PSYCHOSOMATIC CONSTITUTION AND STRESS

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Abstract. A thorough knowledge of the patient's earlier life is required for an assessment of the background of Menière's disease. Psychosomatic factors should be given special attention during interviews. For a correct picture to be obtained, the help of a specially trained worker i.e. a psychiatrist or psychologist, should be enlisted. Factors and causal relationships can then be uncovered which remain problems to doctors not thus specialized. The two series dealt with in this study illustrate this clearly. Summarizing the observations made, Menière patients subjected to careful psychic and somatic analysis are found to have a predisposing psychosomatic constitution which under chronic or acute stress, leads to such somatic manifestations as, in their turn, seem to presuppose a certain somatic predisposition—in this particular case evidently in the ear. Chronic stress seems to impair prognosis, a fact not difficult to understand considering that the mode of reaction depends upon constitution, which—obviously—is of fairly permanent nature.

There is division of opinion on the relationship of Menière's disease and stress. The hypothesis that this disease is of psychosomatic origin was studied recently by Watson et al (1967) who compared the Minnesota Multiphasic Personality Inventory (MMPI) profiles of 40 Menière patients and 38 vertiginous controls. Slightly more than a chance number of significant between scale differences appeared, but those found usually favoured the psychosomatogenic hypothesis. Taken as a whole the results produced only minimal evidence for the general hypothesis. The authors stressed the fact that none of the available earlier studies included careful control series, and thus the literatur was unclear and conflicting.

Consideration of Psychic Factors

In dealing with psychic factors associated wit a somatic disease, the matter can be approached along various lines. It is possible t look at it from the point of view of stress an consider the correlation between life stress an somatic diseases. Care must then be exercise not to take the stress concept too superficially since this may lead to quite erroneous opinion on the dynamics of such diseases. Silverman (1968) quotes Engel's definition of stress as follows. "Psychological stress refers to all processes, whether originating in the external environment or within the person which impose a demand or requirement upon the organism the resolution of handling of which require work or activity of the mental apparatus before any other system is involved or activated" and he goes on to state: Psychological stress derives particularly from interaction with other human beings and from reaction to the social, economic, and cultural settings which man has created. If stresses from these sources are sufficiently intense and sustained, it may be assumed that any individual will react strongly. Most stresses, however are those which are associated with the unpredictable vicissitudes of everyday life and are minor conflicts. At

imes, what on the surface appears to be insignificant or trivial stress or is not even considered as a cause of tension by the casual or uninformed observer may have far reaching symbolic meaning for the individual involved, acting to disrupt the psychophysical equilibrium and perhaps setting the stage for development of illness in which physical symptoms appear prominently." The same author describes various forms of stress—even success may become a stress because of the guilt feelings it produces or because a person tending to have feelings of inadequacy will experience as stress the need to keep up a performance level required for success. Special attention is called by Silverman to the following type of stress: "A particular form of psychological stress has come under scrutiny in recent years. It has been more or less closely related to the onset or exacerbation of a large variety of physical illness. The stress is associated with the actual or threatened loss of an object emotionally significant for the individual concerned. ... There may develop an inability to maintain necessary and sufficient object relationship by substitute means. It is postulated that when this culminates in feelings of helplessness and hopelessness, a profoundly negative effect is exerted on bodily processes."

The effect of stress on the origin of various somatic diseases has been extensively studied. It seems that, in the case of specific somatic manifestations, a more important part is played by biological than by emotional factors. A certain organic weakness, vulnerability, must be presupposed in the parts of the organism affected by the somatic disorder. As indicated by the above, stress situations cannot be viewed according to a set pattern. While to one person an external conflict situation becomes a serious stress causing breakdown, another may experience the same conflict as a stimulating challenge to more efficient action.

Phenomena leading to disease can also be approached by attempting to outline features in a patient's whole personality pattern, such as integration of the personality, emotional response, human relations, and dynamics of drives. Such a study is concerned with the whole person as moulded by structural factors and life experiences, keeping in mind also that man partly chooses his own environment and experiences. Actually it is possible to chart the traits of human personality along similar lines, whether dealing with "total personality" or "various life stress situations".

In an earlier report on the psychological aspects of Menière's disease Siirala and his co-workers (1965) concluded that this disease can develop on the basis of emotional stress in patients having a certain psychosomatic constitution. Studies of the problem "Psyche and Morbus Menière" have been continued and some further observations have emerged.

The present study

The study was carried out in two phases. The earlier material consisted of 133 Menière patients treated at the Otolaryngological Department of Helsinki University Central Hospital during the period 1957–66; the later series of 33 patients treated during 1966–67 who were subjected to conventional otologic-audiologic-otoneurologic testing and, in addition, to *thorough psychological testing*. The data on the earlier series are from the case records based on examination and test results and on a follow-up inquiry.

Menière cases treated in 1957–66 (133 patients)

The study aimed at gaining information on the development of the disease and its possible correlation with other factors, mainly so-called stress. The patients accepted into the series presented typical Menière symptoms: sensorineural hearing loss, attacks of vertigo and tinnitus. They were subjected to the following examinations: pure tone and speech audiometry, recruitment tests, and nystagmography for demonstration of vestibular function.

Criteria for the stage of disease were: vertigo, deafness and tinnitus. Other parameters

were smoking, salt intake, allergy sleep and work.

Treatment consisted basically of a low salt diet, rest, the advice not to smoke (followed by most) and to avoid physical and mental strain. In addition, most patients received sedatives, tranquillizers and sleeping pills, nicotinic acid, antihistamines, soda therapy procaine histamine, dihydroergotamin, diuretics and cortisone. The study being designed mainly to discover the part played by stress in Menière's disease, special attention was devoted to factors indicating the presence of psychological stress.

Available data on the nature and amount of stress in Menière's disease are variable. These questions can only be thoroughly clarified if the patients are interviewed in great detail by a psychiatrist or psychologist. (Indeed this was done in our later series of 33 patients.) Because the case records and follow-up inquiries revealed definite stress factors in 56 of the 133 cases of the earlier series, a comparison was made (Table I) as regards development and stage of the disease, between these 56 patients and the 77 patients whose record suggested no stress whatever

It is seen that 42% of the Menière patients studied by follow-up inquiry were or had been exposed to stress. Study of the development of their disease, taking vertigo as chief criterion, showed that the stress group included 21% (36% of the total of cured) who got rid of symptoms of vertigo, the same figure in the stress-negative group being 27% (64% of total cured). The stress positive group further included 50% whose vertigo improved (39% of total of improved) against 57% (61%) in the stress-negative group and 29% who were not helped by treatment and still suffered from vertiginous attacks (57% of total not cured). In the stress-negative group there were 16% (43% of total not cured) who continued to suffer from vertigo

Table I *Number and status of stress-positive and -negative patients on the basis of follow-up inquiry*

		Symptoms of vertigo		
		Cured	Improved	Not cured
Exposed to stress	56 (42)	12 (21)	28 (50)	16 (29 %)
Not exposed to stress	77 (58 %)	21 (27)	44 (57)	12 (16 ,,)
	133	33	72	28

Table II

		Surgically treated patients		
		Cured	Improved	Not cured
Exposed to stress	16	3	9	4
Not exposed to stress	14	5	7	2
	30	8	16	6

Sixteen of the patients exposed to stress were treated surgically in most cases by a destructive operation on the labyrinth, performed through the oval window. Of the stress-free patients, 14 were similarly treated. Table II presents the changes after operation.

The total number of operated patients being as low as 30 it is difficult to say anything definite about their prognosis. Both the conservatively and the surgically treated patients show a difference in favour of the stress-negative cases, but the difference is not significant. As far as the surgically treated cases are concerned it should be taken into account that operation is frequently so successful a treatment that the vertigo disappears even though stress persists.

Menière cases treated in 1966–67 (33 patients)

During the period 1966–67 33 patients were tested by a psychologist with the particular purpose of uncovering features and events in the lives of the Menière patients and their personality patterns

The purpose of this investigation was to check the results of the selected material reported in the previous paper (Siirala et al. 1965) by studying a case material that was entirely unselected, actually each Menière patient admitted to the Clinic between autumn

1966 and the end of *1967* was subjected to exactly the same psychological tests. When asked to attend psychological tests, all these patients agreed to do so, and evidently with pleasure because this brought variety to the day-to-day hospital routine

Case material The patients totalled 33 23 women and 10 men. The women varied in age from 20 to 70 years and the men from 31 to 61 years. The majority were in the forties" median age was 43 for the women, 45 for the men. Of the 23 women, 17 were married, 3 single, *1* divorced, and 2 widows The men were all married. Most of the patients had children, only 2 men and 4 women were childless. As regards school attendance it is c. interest that only 6 of the total 33 had completed middle school (lower secondary) and only 3 of these had gone on to higher level. None had received academic education. The great majority had attended primary school, the an old type of ambulatory school 2 the school of the Disabled Foundation. The limited school education is noteworthy in view of the fact *that the majority of the patients had a* fairly high intelligence level as judged both by interview and Rorschach test. Five of the men were regularly employed, but one of these said he had often had to change jobs. At the time f the testing, one man was unemployed, evidently through no fault of his own. One said he could only manage to do a little work, 3 had stayed at home for good In the case of the women, fitness for work was more difficult to evaluate, since for most women the home is the usual place of work. Ten of them had outside employment, the rest worked in the home. The impression was gained that most of these could cope with their housework fairly well. The biographical data of the patients will be considered below

This series of patients we consider of special interest in that the external events of their lives as well as their mode of reacting were surprisingly uniform—according to both the interviews and Rorschach tests. We therefore wish to list several points characterizing the "typical Menière patient" though most of these are probably common to a great number of people with a tendency to psychosomatic diseases

Biographical features. One point was touched upon above, viz. limited schooling. A great number stated spontaneously and obviously affectively that they would have wished to continue at secondary school or become skilled workers in some field, but better schooling was prevented in most cases by economic difficulties, in 2 cases by illness at school age Many said that their elementary school teachers had urged them to have further education. The patients opinion of their own qualifications for study is supported by the fact that the Rorschach test showed a good level of intelligence In addition, the children of most patients later got on well at school and were successful in life Most of the patients had not got rid of the frustration caused by fairly poor achievement in life they felt they had abilities, at least intellectual abilities, to achieve something better

A great number had suffered traumatic experiences *in childhood, for instance through* the death of one parent. The mother or father of 11 of the 33 patients had died early Four patients said that their fathers had been alcoholics The father of one was in prison. According to 3 patients, one parent had been extraordinarily severe A further experience was displacement. Five said that after the war they had had to move away from their homes in Karelia to a strange part of the country This was often associated with conflicts between husbands and wives coming from different provinces and therefore differing in nature. Three of the men stated that taking part in the war had been a traumatic experience Two had moved from one locality to another at the time Menière's disease set in.

Various Illnesses Close inquiry as to the illnesses (other than Morbus Menière) the patients had suffered from revealed a great number of conditions generally regarded as psychogenic. Only two of the total of 33 patients stated that they had always been healthy F

had had only minor illnesses. One patient, it is true, said that her peptic ulcer was cured when Menière symptoms started. The following diseases were reported: allergy, migraine or other severe headache, gallstone pain, heart symptoms, peptic ulcer, asthma, colitis, obesity. A high proportion of the women showed symptoms indicating sex hormonal imbalance. The patients' age should be noted: many of the women seemed to be approaching the menopause.

A feature obviously typical of these patients seems of interest: On being asked about other diseases, the first answer was almost invariably "nothing of importance". But in the course of further talks they liked to speak about their diseases and described them in detail. This in its way reflects their psychological type: they all seem to have poor insight, they are practically incapable of recognizing their own impulses and difficulties. At the same time they are greatly dependent upon the acceptance and interest of other people—they wish to be "especially interesting cases". This should be taken into account in general when interviewing people with tendencies towards psychosomatic disorders. Especially in test situations where little time is allowed, such people tend to be [illegible]*ted and often give negative or fortuitous* [illegible]. This showed itself by such expressions as "Surely people don't think I'm mad because I'm sent to a psychologist" but later all patients expressed their satisfaction that for once someone listened to them and their troubles. Clearly many patients experienced the test situations as highly therapeutic—several said that they felt relieved because "they had been able to speak of their troubles". Obviously there is a considerable need for psychotherapy in Menière's disease also, and this appears to be yet another channel through which these sufferers might be helped.

Results of Rorschach tests. These were astonishingly similar for the great majority of the patients. The most remarkable features were: poor insight, patients not able to recognize their own impulses and troubles, absence of a spontaneous warm and affective responsiveness to other people and environmental stimuli, overambitiousness, frequently of traumatizing degree and associated with inferiority feelings. One might speak of poorly integrated persons, having weak egos, who cannot cope with their difficulties and respond both to situations causing agony and to inward impulses of their own—by running away from them. Intelligence, however, seemed to be good in almost all cases. Slightly healthier test results were obtained in those exceptional cases in which illness in youth had resulted in extremely great practical difficulties (e.g. Mrs 23, severely disabled by polio, who tried to take care of her small child and do her housework in very poor housing conditions; Miss 20 whose face had been disfigured by lupus when young). Expressed in Rorschach terms, the following emerged. An extremely high W% (in many cases 100%), level of replies average or better. Colour replies were entirely lacking in several cases—a few gave only one or two replies, expressing explosiveness, fires, blast etc. Various "shading" answers were fairly common. The popular M replies to the III-table were obtained from most patients, but other M-replies were infrequent. Anatomy replies, often sex coloured, were common. *Childish fairytale-like* replies occurred in great number (bunnies, Santa Clauses, Donald Ducks).

CONCLUSIONS

From the above it is evident that a constitutional predisposition is the fundamental cause of Menière's disease. The report of Watson, Barnes, Donaldson and Klett, based on thorough personality inventories, indicated that there was only minimal evidence in favour of a psychosomatic etiology. The present study showed that 58% of the series not subjected to psychological testing were stress-negative and their prognosis was slightly but not significantly better compared with the stress-positive patients. On the other hand, the patients tested psychologically by interviews and Ror-

schach method, were found to show features very much along the same lines and could be assumed to be highly predisposed to psychosomatic reactions. Stress factors were noted in a great number but they varied in nature and amount and had occurred during various phases of life.

The authors find that, in charting psychic factors associated with somatic diseases, it is important to study the essential features of the patients whole personality pattern, the level of integration of their personality the way in which they respond to stimuli, the sum of their life experiences. Only against this background can the more direct effects of various stress factors on the origin of disease be evaluated

In this context it should be remembered that there are many people with weak egos who do not get Menière s disease, that there are countless numbers exposed to severe stress who remain healthy or get some other disease. Here evidently the role of the biological structure should be taken into account. In the presence of the psychic structure and external stress required for psychosomatic reactions to develop selection which determines the type of consequent disease, seems to depend mainly upon the organic properties of the patient. In other words, the patients who get Menière s disease must be assumed to have inner ears anatomically and physiologically predisposed to Menière s disease Obviously psychic factors decide whether the disease breaks out at all, and if so, how frequent and how severe the attacks are

A comparison between the 1965 series, in which a selected group of Menière patients were studied by a psychologist, and the 1966–67 series, unselected and studied by another psychologist, showed that, in both of these, the psychosomatic constitution played an important part in the outbreak of Menière s disease. While in the selected series stress was a direct factor there was no direct temporal relationship between stress and Menière attacks in the unselected series This is explained by the fact that the former consisted only of selected cases whose disease had been provoked by stress factors

ZUSAMMENFASSUNG

Um den Hintergrund der Menière schen Krankheit richtig beurteilen zu können, ist eine gründliche Kenntnis des früheren Lebens des Patienten erforder lich. Beim Aufnehmen der Anamnese und bei den Unterredungen mit dem Kranken sollte auf die psychosomatischen Faktoren ganz besonderes Gewicht gelegt werden. Um ein zuverlässiges Bild zu erhalten, müsste eine speziell ausgebildete Person, m. a. W em Psychiater oder Psychologe herangezogen werden. Auf diese Weise lassen sich Faktoren und zufällige Zusammenhänge aufdecken, die dem diesbezüglich nicht spezialisierten Arzt schwerlösliche Probleme bleiben. Die zwei Gruppen, mit denen die vorliegende Unter suchung sich befasst, zeigen dies ganz unverkennbar Zusammenfassend lässt sich folgendes sagen: Es stellte sich heraus, dass die Patienten mit Menière scher Krankheit, die einer sorgfältigen psychischen und somatischen Analyse unterworfen wurden, eine prädisponierende psychosomatische Konstitution besassen die bei chronischem oder akutem Stress zu solchen somatischen Manifestationen führt, die ihrerseits wiederum eine gewisse somatische Prädisposition vorauszusetzen scheinen, in diesem speziellen Fall ganz offensichtlich das Ohr betreffend. Chronischer Stress scheint die Prognose zu beeinträchtigen, was leicht verständlich ist im Hinblick darauf, dass die Reaktion von der Konstitution abhängt, die offensichtlich ziemlich beständiger Natur ist.

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PERSTIMULATORY SUPRATHRESHOLD ADAPTATION

II Conductive Deafness

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Abstract Perstimulatory suprathreshold adaptation was studied on 30 otosclerotic and 40 chronic ears, using the decrease in loudness level of a pure tone as a criterion. The comparison tone was interrupted the pulses and free intervals were each of 200 msec duration. Measurements were carried out at 60 dB sensation levels for frequencies 1 000–4 000 Hz. The prestimulatory balance levels were higher than in normal hearing ears. Adaptation was of equal amount as in normal ears and could be classified into three different types. In conductively deaf children adaptation could be demonstrated only exceptionally

Suprathreshold auditory adaptation may for the most part be a cochlear phenomenon to a lesser extent it may occur in the central auditory pathways (Keidel 1958 Keidel et al., 1960) Thus, theoretically adaptation in conductive deafness is of equal degree as in normal hearing subjects, although certain factors in middle ear sound transmission may modify the results Only a few pertinent studies have been published hitherto, and the technique employed has not been faultless.

In unilateral conductive deafness, Hood (1950) recorded an adaptation which, at 70 dB SL and 2 000 Hz, was equal to that found on the healthy side Balancing was made with a continuous comparison tone in the healthy ear for 3 min, thus, adaptation in the other ear was not really measured. Palva s (1955) study included 18 conductive ears tested at 80 or 100 dB above normal threshold for frequencies between 500 to 4 000 Hz. Adaptation in 3 min, measured with 15 sec continuous tone balance, was of the same magnitude as in the normal-hearing subjects tested with the same technique Pestalozza (1953) and Bosatra (1957) used Hood s technique and found normal adaptation values (30% loudness loss) in conductive deafness All these results were modified by varying amounts of adaptation occurring in the comparison ear

MATERIAL AND METHOD

The material consisted of two groups of conductively deaf patients, one with chronic otitis, the other with otosclerosis. Age varied in both groups from 20 to 45 years and averaged 35.0 and 37.5 years, respectively The former group consisted of 40 patients in 19 cases infection was unilateral and in 21 bilateral. In 30 otosclerotic patients—all later operated—the disease manifested itself in both ears in 20 and in one ear in 10 cases. In addition 26 young (9–17 years) conductively deaf patients were tested (22 chronic and 4 otosclerotic ears).

All test subjects had bone conduction values better than 20 dB (ISO-standard) at 250–4 000 Hz. Speech reception thresholds correlated with the pure tone air conduction values, and speech perception exceeded 90% in all cases.

Air and bone conduction audiograms were

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measured by the usual descending–ascending technique using a Madsen Model OB 60 audiometer. Speech thresholds and discrimination were determined for each patient as described by Palva (1952). Loudness recruitment was measured by the Fowler or Reger method, or utilizing the amplitude of the threshold recording in a self-recording audiometer.

The apparatus for measuring adaptation was identical with that described in detail earlier (Kärjä, 1968). Adaptation was measured at one to three suitable frequencies between 1 000 and 4 000 Hz using stimuli 60 dB above air conduction threshold. In bilateral cases only one ear was studied.

Determination of air conduction thresholds with interrupted signal by Grason-Stadler Audiometer Model E 800 for the frequency tested was followed by prestimulatory balancing. An interrupted pure tone stimulus was presented to the test ear at desired sensation level while a similar comparison tone was fed to the control ear. When the subject had recorded the balance for 30 sec, an adapting, i.e. continuous, stimulus was introduced into the experimental ear for 3 min. Balancing with an interrupted 200 msec tone was continued throughout the stimulation. Recovery of the threshold in the test ear was recorded for 60 sec after cessation of stimulation. When measurements were made at several frequencies, the interval between adapting stimuli was at least 5 min. Adaptation in dB was calculated from the difference between the adaptation curve and the prestimulatory balance level.

RESULTS

In the groups with otosclerosis the values at 2 000 and 4 000 Hz are means for 20 patients, in chronic ears at 2 000 Hz for 26 patients and at 4 000 Hz for 23. Ten ears were tested in both groups at 1 000 Hz.

Prestimulatory balance

Prestimulatory balance levels (Table I) tended to be higher in both otosclerotic and chronic

Table I. *Prestimulatory balance levels*

	Frequency (Hz)					
	1 000		2 000		4 000	
Diagnosis	Mean	S.D.	Mean	S.D.	Mean	S.D.
Chronic ears	61.3	11.2	69.0	8.7	68.0	6.9
Otosclerosis	64.6	9.6	62.0	10.8	63.2	6.9
Normal ears (Kärjä 1968)	58.9	10.0	57.4	11.4	57.1	8.2

ears than those recorded in normal ears. The differences were significant ($P < 0.05$) in both groups at 4 000 Hz and in chronic ears at 2 000 Hz. The balance levels were clearly affected by the threshold differences between the test ear and the control ear (Table II). If the difference was less than 10 dB (or the test ear better) the results were comparable to normal (Kärjä, 1968); when the threshold of the test ear was 10–30 dB (or more) poorer than that of the comparison ear, balancing resulted in significantly higher levels than in the normal group.

The amplitudes of prestimulatory balance tracings are shown in Table III. They are significantly greater than the threshold amplitudes (excepting otosclerotic ears at 2 000 Hz). There were no differences between the two conductive groups. As related to normal material the balance amplitudes were wider in the otosclerotic group at 2 000 Hz and in chronic ears at 1 000 Hz.

Table II. *Prestimulatory balance levels as a function of difference between the thresholds of test and comparison ear (the two conductive groups combined)*

	Difference between thresholds (dB)					
	<10		10–30		>30	
Freq. (Hz)	Mean	S.D.	Mean	S.D.	Mean	S.D.
2 000	53.2	10.3	70.0	6.3	75.6	8.9
4 000	58.9	10.3	67.5	6.9	70.5	7

Table III *Excursion widths of prestimulatory balance tracings compared with threshold amplitudes of control ear*

	Frequency (Hz)											
	1 000				2 000				4 000			
	Thr		Bal.		Thr		Bal.		Thr		Bal.	
Diagnosis	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D.	Mean	S.D.
Chronic ears	7 1	1 4	8.4	1.0	6.6	1 4	8.4	2.5	6.2	1.3	7 6	2.5
Otosclerosis	6.1	1 1	7 6	2.0	7.0	1.9	8.0	3.9	6.1	1.6	8.2	2.4
Normal ears (Kärjä 1968)	5 6	1.9	6.5	2.2	5.2	1 7	6.6	2.3	5 0	1.2	6.6	3.1

Adaptation

Perstimulatory suprathreshold adaptation in conductive deafness was characterized by large individual differences, as in the case of normal subjects (Table IV) There were three adaptation types, the same as in the normal material. Type I, with no adaptation or adaptation less than 10 dB included 2 (6 7%) patients from the otosclerosis group and 2 (5 0%) chronic ears. Type II slowly developing adaptation, was found in 16 (53.3%) otosclerotics and in 23 (57.5%) patients with chronic ear disease and the same figures for Type III, rapid growth of adaptation, were 12 (40%) and 15 (37.5%) respectively The mean adaptation values ten- to be greater in both conductive groups in normal subjects (Figs 1–3) The dif s were not significant, however

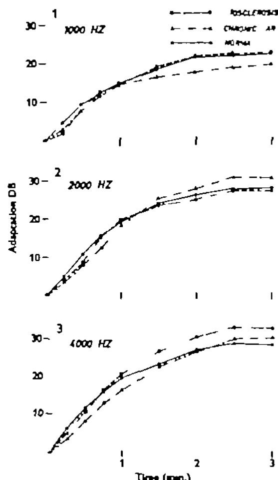
Adaptation was measured on 10 patients younger than 15 years suffering from chronic middle ear inflammation. Only one boy aged 14 years, experienced a loudness loss of the adapting stimulus, amounting to 34 dB at 4 000 Hz. Among 12 patients aged 15–17 years, adaptation of more than 10 dB occurred in as many as 8 cases. In 4 operatively verified young otosclerotic patients the case was similar 2 were 12 years old and showed no adaptation. The other two were 15 and 17 years old, adaptation (50 dB at 2 000 Hz and 43 dB at 4 000 Hz) could be measured in the latter only

The widths of the adaptation tracings are presented in Table V The values for adapted ears in both groups were statistically the same as the means for prestimulatory balances. In addition, the differences between the conductive groups were not significant.

A change in quality of the pure tone stimulus was the rule in conductive deafness also it became lower than the comparison tone or

Table IV *Perstimulatory suprathreshold adaptation for 1 000 2 000 and 4 000 H at 60 dB SL for 3 min*

	Chronic ears						Otosclerosis					
	1 000		2 000		4 000		1 000		2 000		4 000	
Time (sec)	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D.
15	3.0	6.3	3.5	6.4	3.6	7 4	1.9	4.3	4.1	7.2	5.0	7.2
30	6.7	9 1	8.0	10.1	8.2	11.3	8.2	10.8	8.5	8.7	10.8	12.6
45	12.0	17 7	12.3	12.7	13.0	13.4	12.9	15.7	15.5	11 6	16.6	15.3
60	14.7	20.1	18.0	16 7	16.5	14.0	14 1	17.3	20.0	14.7	20.9	16.4
90	18.0	22.6	25.8	18.4	23.0	14 4	19.2	21 6	23.7	17 7	27.2	15.[illegible]
120	16.5	23.2	28.0	18.4	27.0	14 6	21 7	22.6	24.7	18.7	31 1	14 1
150	18.5	24.1	31.5	18.4	30.6	15.0	22.4	22.1	27.6	18.7	33.8	12.7
180	19.9	24.2	31.0	18.3	30.6	13.9	22.9	22.4	27.5	18.6	33.2	13.0



Figs. 1–3. Perstimulatory suprathreshold adaptation at 60 dB SL for frequencies 1 000, 2 000 and 4 000 Hz.

resembled noise. In otosclerotic patients the change took place at 2 000 Hz in 70% of the cases, at 4 000 Hz in 85%. In chronic ears the figures were 70% and 90% respectively.

Table V. *Excursion widths of adaptation tracings for fully adapted ears*

Diagnosis	Frequency (Hz) 1 000 Mean	S.D.	2 000 Mean	S.D.	4 000 Mean	S.D.
Chronic ears	8.0	1.8	9.9	3.1	9.7	3.4
Otosclerosis	8.5	2.7	9.5	3.4	9.2	3.6
Normal ears (Kärjä 1968)	7.1	2.6	7.0	2.6	6.8	2.4

Table VI. *Poststimulatory threshold shift 1 min after cessation of adapting stimulus*

Diagnosis	Frequency (Hz) 1 000 Mean	S.D.	2 000 Mean	S.D.	4 000 Mean	S.D.
Chronic ears	3.8	4.0	4.5	5.7	5.5	4.4
Otosclerosis	4.7	5.3	4.0	3.1	5.0	4.1
Normal ears (Kärjä 1968)	4.0	2.6	4.0	3.2	3.3	[illegible]4

Poststimulatory threshold shift

Table VI shows the threshold values of stimulated ears 60 sec after cessation of the adapting stimulus, measured with interrupted test tone. They were of the same order as in the normal material. The amplitudes also did not differ from prestimulatory values, a finding seen in the normal group too. However, the excursion widths of both the prestimulatory and poststimulatory threshold tracings tended to be larger than in normal subjects.

DISCUSSION

On the basis of the experience gained in testing normal group subjects (Kärjä, 1968) the measurements were carried out at 60 dB SL and for the frequencies 1 000–4 000 Hz. The otosclerotic and the chronic ears were found to behave similarly under these test conditions and values comparable to normal were generally found, the most noticeable exceptions being the higher prestimulatory balance levels in the test groups (Table I). The highest balance levels occurred in patients whose threshold values showed the largest differences, the control ear being healthy. If the threshold values were overlapping, the prestimulatory balance levels were the same as in normal material (Table II). The reason for these differences is somewhat uncertain. At levels 60 dB above the air conduction threshold of the impaired ear with normal bone conduction cross-hearing cannot be avoided when ordinary ear

phones are used. Summation phenomena, how ever cannot explain the results. A possible factor may be the recruitment phenomenon in conductive ears, as demonstrated by Anderson & Barr (1966) who used the Fowler test and the stapedius reflex test in cases of unilateral conductive hearing loss. In the present study a partial recruitment was recorded with the Fowler or Reger technique in 10 otosclerotic ears (33 %) and in 23 chronic ears (58 %) and the lesion was unilateral in the majority of cases. The growth of loudness in the ear under test, being more rapid than normal would require added intensity in the comparison ear for equal loudness balance Furthermore, Anderson & Barr found that the degree of recruitment increases with the severity of the hearing loss

Adaptation in conductive deafness and in normal ears proved to be of equal amount and the distribution into three adaptation types occurred in the same proportion. In addition to 1 000 2 000 and 4 000 Hz, a number of experiments were made at lower frequencies and at 40 dB SL, the results did not differ from normal adaptation values These observations are in agreement with the results of other investigators even though the values with the sent technique are higher this is due to the that adaptation in the control ear is voided Adaptation in young patients with conductive deafness was very rare, similarly as in normally hearing children, and it could be demonstrated only in a few older patients in the age group under 15 years.

The two conductive groups chronic ears and otosclerosis, did not differ in the threshold excursion widths, prestimulatory balance, and adaptation tracings. Compared with normal ears, the values for the conductive ears tended to be slightly larger though the difference was not of statistical significance The reason may be that the normal subjects were more familiar with the recording technique, as pointed out earlier by Palva (1957)

ZUSAMMENFASSUNG

Perstimulatorische überschwellige Adaptation wurde an 30 otosklerotischen und 40 chronisch entzündeten Ohren, Verminderung der Lautstärke-empfindung als Kriterium, gemessen. Der Prüfton bestand aus 200 Millisekunden langen Impulsen und ebenso langen Intervallen. Die Messungen wurden bei 60 dB über den Luftkonduktionsschwellen für die Frequenzen von 1 000–4 000 Hz gemacht. Die prestimulatorischen Vergleichungspläne waren höher als die der Normalhörenden. Adaptation war ebenso gross und verteilte sich auch in dieselben drei Gruppen wie beim Normalmaterial. Bei Kindern konnte man Adaptation nur dann zeigen, wenn sie sich exceptionell ereignet.

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THE HYPERACTIVE VESTIBULAR RESPONSE

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Abstract A hyperactive nystagmic response following routine vestibular stimulation has long been recognized. Central pathology was suspected as responsible for increased postcaloric or postrotatory nystagmus and more recent experimental studies confirmed hyperacti nystagmus after lesions in the cerebellum ere created. In order to utilize the hyperactive nystagmus response as an abnormal clinical sign, it was essential to establish criteria for this phenomenon. In 67 normal subjects weak and strong caloric stimulations were performed and by measuring nystagmus frequency at the culmination a wide range of responses was obtained. Using these data as base line, three groups of hyperactive responses were classified in 1 700 patients. Group A exhibited nystagmus intensities never encountered in the control subjects. Group B represents an increased nystagmus activity at the range which has been found only exceptionally in normals (in 3 out of 133 normal ears). Group C comprises the category of patients in which responses can be considered as pathologically increased although 10% of the normal subjects showed such excessive frequencies. Therefore, our patients in this border line area may be considered potentially hyperactive. In unilateral occurrence of hyperactivity the pathological designation is further enhanced. The postrotatory responses were similarly classified. A distribution by diagnosis of our 68 patients who had hyper curve responses demonstrates variety of central bnormalities Hyperactivity was found in few instances with peripheral pathology such as otosclerosis or vestibular neuronitis, but the hyperactivity was found on the contralateral side. In small group of the patients no pathological findings could be obtained except for exaggerated caloric nystagmus. These patients were overly anxious and concerned about their complaints of dizziness and uncertainty There were 7 out f 68 who had bilateral hyperactivity and were diagnosed as having psychosomatic disorders The study led to the conclusion that the hyperactive vestibular responsiveness is a clinical entity It can be detected by standard stimulation and reliable nystagmography It was found to occur in wide variety of central nervous system disorders and occasionally in neurotic, psychosomatic patients.

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Ever since nystagmus could be elicited by rotatory or caloric stimulation, an unusually strong or violent nystagmic response has oc casionally been encountered. A weak appear ance or absence of the nystagmic response has been identified as a defect of the vestibular function. By contrast, an extremely forceful eye movement reaction was recognized first as an increased vestibular excitability Such a conclusion was reached when the post-stimulatory nystagmus was prolonged considerably with or without an additional increase in the amplitude and frequency of the beats. A similar judge ment was made when the average stimulus caused an unexpectedly strong reaction of nausea, vomiting, perspiration, pallor etc.

The causation of such increased vestibular sensitivity was theorized by Brunner (1924) with three possible reasons. (1) a less than average cupular deflection elicits an average response (2) the peripheral impulse reaches the center faster than average (3) a qualitatively and quantitatively identical stimulus cre ates a stronger reaction than average.

It was suspected long ago that prolonged nystagmus responses might be caused by some central nervous system pathology and it w soon observed that diseases of the cer

often were associated with hyperactive nystagmic responses. Ruttin (1916) related the phenomenon to lesions of the vestibulo-cerebellar pathways, and Bauer (1916) and Leidler (1918) thought that a paralyzed vestibulo-cerebellar connection allows all the neural energy to activate the eye muscles with greater force. Hyper active nystagmus response was found in cere bellar tumors and Benesi & Brunner (1921) postulated that hyperactive responses occur only in cerebellar tumors when the intracranial pressure is increased. Scattered observations were reported of hyperactive vestibular responses in various diseases of the central nervous system In one patient with syringobulbia (Brunner 1924) and in one case of hereditary lues (Boeters, 1914) such a reaction was found. Further in cases of traumatic neurosis the prolonged or stronger nystagmus was attributed to the "generally increased excitability of the entire nervous system" (Brunner 1924) In 1942 Spiegel & Scala could produce experimentally a markedly enhanced rotatory nystagmus after cerebellar lesions were created in cats. A basic experimental work of Fredrickson & Fernández (1964) has proved that damage to the nodulus with varying involvement of the vestibular nuclei and the floor of the fourth ventricle is associated with a hyperactive vestibular re

in addition to postural nystagmus and

without ataxia. A conclusion

ould be drawn that the increased nystagmic activity is due to a release of vestibular centers from cerebellar nodulus inhibition. These findings were in agreement with the clinical ex perience of Riesco-McClure (1964) who found hyperactive nystagmic responses in patients with cerebellar or midline lesions in the poste rior fossa. It was also pointed out tentatively that increased vestibular responsiveness has no localizing value unless it is associated with spontaneous and positional nystagmus, disequilibrium, disrhythmia or other cerebellar signs. A similar report was presented by Alberanz-Mangabeira (1966) finding vestibular hyper reflexia with fast vestibular habituation in 5 out of 7 cases having lesion in the brain stem.

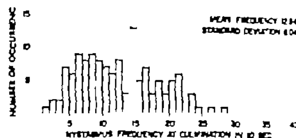


Fig 1 Distribution of culmination frequencies of weak caloric responses in 133 normal ears.

When discussing the phenomenon of an increased nystagmic responsiveness following vestibular stimulation, the first question to be answered is what would constitute such a reaction, or what are the criteria for a hyper active vestibular nystagmus. Hyperexcitability has also been mentioned in patients with not so much a nystagmus increase, rather an unusually strong response of autonomic nervous reactions of nausea, vomiting, etc. These two clinical manifestations have different origins and significance and more commonly appear independent from each other Hyperactive nystagmus may be a sign of central nervous system pathology affecting vestibulo-cerebellar connec tions whereas increased vertigo and vagal reac tions are effects of autonomic dysfunction.

Increased caloric or rotatory nystagmus is so obvious that it prompted Neumann (1914) to use the term nystagmus clonus" The earlier references were documented primarily by a prolonged duration of the nystagmus Today when nystagmography is available, reliable parameters of induced nystagmus can be utilized. In our experience frequency at the culmination of caloric nystagmus offers a reliable and simple method of assessing nystagmus intensity (Torok, 1948 1962)

It seemed reasonable to study the responses obtained through meticulously standardized stimulation in a greater number of subjects with no diseases in general and of the cochlear and vestibular systems in particular In this investigation, conducted earlier with other objectives, 133 individual caloric stimulations were carried out with two different stimuli (Torok, 1969).

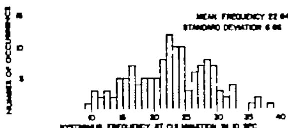


Fig 2 Distribution of culmination frequencies of strong caloric responses in 133 normal ears.

A weak" stimulus was represented by inject ing 10 cm³ of 20 C water in 5 sec and a strong" stimulation with 100 cm³ of water of the same temperature applied for 20 sec. Photo-electric nystagmography was employed, the subjects were tested with eyes open in dark ness (Nykiel & Torok, 1963) The culmination frequencies spread over a wide range be tween 3 to 29 nystagmus beats at the culmination for the weak stimulation (Fig. 1) and 10 to 39 frequencies were counted when the strong stimulation was applied (Fig 2) By eliminat ing a few excessively low and high frequency responses, the 90% of all ears tested fell be tween 5 to 22 for the weak caloric and 15 to 33 for the strong stimulation

The range of the normal postrotatory nystagmus was evaluated on the basis of another study conducted on normal test subjects (Torok, 1969) Using a 45 /sec steady angular velocity until all perrotatory nystagmus had subsided, a sudden stop (between 200–300 msec) constituted the stimulus. The postrotatory nystagmus was again registered with photo-electric goggles (I.e., open eye in darkness) and the frequency of the beats was counted for the first 5 sec time periods. Thirty four tests were conducted in 17 individuals. The mean of nystagmus frequency for the first 5 sec was 5 7 5 4 3 Considering the standard devia tions obtained for each of the five time periods, the frequency values acceptable as the latitude of the average became: 13 to 38 beats during the first 25 sec of the postrotatory nystagmus.

These results and calculations served as guidelines to establish criteria for hyperactive responses

In our records of patients tested between 1966 and 1968 68 cases have been found with higher nystagmus frequency values than encountered in our normal population. Of this group, 37 exhibited a bilaterally increased re sponsiveness and in 31 patients the hyperactive nystagmic response was restricted to one sid. All these patients have been tested over a relatively long period (3 years) with various clini cal diagnoses. Some of them were severely ill when all and otherwise-routine test procedures could not be performed. The available data are therefore not completely uniform, though the various types of test have all been executed on a standardized basis. All patients had caloric tests. In the majority a small stimulation (10 cm of 20 C water in 5 sec synnged in the pessimal head position and nystagmus recorded after 60 sec in the optimal head position i.e canal horizontal (Veitz)) and a large stimulation (100 cm³ of 20 C water in 20 sec with nystag mus registered promptly in optimal, canal horizontal position) were performed. In a certain number of cases, when the weak stimulation elicited an excessively strong response, the strong stimulation was not attempted. Even greater caution was applied with rotatory sti mulation. In only 52 of the 68 selected cases was a rotatory test added to the thermic stimulation Attempts have been made to correlate all the available data and information.

The wide range of physiological responses to the various stimulations makes ascertaining a hyperactive response difficult. It was felt practical to classify all our hyperactive reac tions into three groups. Group A includes those patients exhibiting a culmination frequency of 30 or higher after weak caloric stimulation and 42 beats or higher culmination for the strong irrigation. Group B spans 24–29 frequencies for the weak and 36–42 beats for the strong stimulation. Group C varies between 18–23 for the weak and 30–36 for the strong caloric effect. While it is understood that such classification is arbitrary it pro ed meaningful in clinical use. Group C represents test results which appear to be exaggerated, though these freq

values have been encountered occasionally in normals Because only about 10% of normal subjects had shown such excessive frequencies, our patients in this borderline area may be considered potentially hyperactive Group B type cases produced large nystagmus intensities which occurred in only 3 of 133 normal ear stimulations with weak stimulus and only 4 times in the same number of tests with the strong effect. These cases, therefore, can be considered more definitely pathological Group A patients have a strong abnormal hyperactive response. No normal subjects have been found to produce such an increase in nystagmus frequency after caloric stimulation. These results are unconditionally pathologic.

A similar attempt was made to classify the postrotatory responses. Only two groups were delineated. Group B with total nystagmus frequency for the first five 5 sec periods following the sudden stop, from 38 to 50 beats and group A, 50 beats and above for the same period. Whereas the figures of group B occurred in about 10% of normals, the increased nystagmic activity classified in group A was never seen in normals and can be considered as definitely pathological.

As our records show many of the hyperactive responses were not concurrent for the different types of stimulation. A general hyperactive responsiveness was less often seen than those where the weak stimulus, or only the strong, created exaggerated responses On other occasions, when both of such responses were increased the hyperactivity was not proportional for the thermic and rotatory effects.

Finally two major categories of hyperactive reaction could be recognized. those cases where

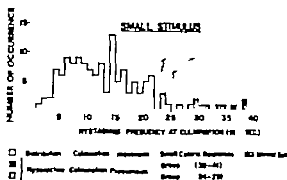


Fig 3 Bilateral hyperactive nystagmus response in 6 patients (52 ears).

a symmetrically increased responsiveness was found, i.e the hyperactivity was bilateral, and an almost similarly large group of patients where our criteria of hyperactive reaction were manifest only on one side

Bilateral Hyperactive Responses

Thirty seven patients in our group exhibited hyperactive nystagmic responses on both sides by the criteria defined above.

In group A 13 patients were subdivided and are recognized as having at least 30 beats in 10 sec at the culmination after the weak caloric stimulation, and the highest so found had 39 culmination frequencies (Fig. 3) For the strong stimulation, the lowest of culmination values was 42 and the highest found was 5 per 10 sec (Fig. 4) (It should be noted that the photoelectric recording system with d.c. amplification is particularly capable of recording such extreme frequencies.) Of 5 in the group rotatory responses were similarly in-

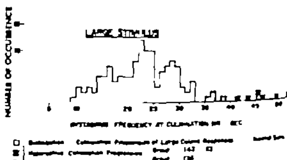


Fig 4 Bilateral hyperactive nystagmus response in 8 patients (16 ears).

For sake of comparison, the duration of all nystagmic responses was plotted in each category of nystagmus intensity The overall average of duration was perhaps higher than in our normal group but no relationship between frequency culmination and total duration was found. In some instances, unusually short duration was measured (40 sec) or it was as long as 3 min. Similarly the amplitude of the nystagmus varied between very small (1 mm) and extremely large (1 mm) irrespective of the frequency count.

Table I. *Bilateral hyperactive responses. Group A*

Diagnosis	No.	Symptoms
1 Central nervous system involvement	4	Ataxia & cerebellar symptoms (2) Ataxia, mitral stenosis, cerebrovascular pathology (1) Severe arteriosclerosis, unilateral sudden deafness (1)
2. Basilar artery insufficiency	2	Paroxysmal postural vertigo (2)
3. Epilepsy	1	
4. Head trauma	1	Postural vertigo and nystagmus
5. Multiple sclerosis	1	
6. Spontaneous ocular nystagmus	1	Congenital
7 Otosclerosis	1	Mixed hearing loss at age 74
8. Psychosomatic disorder	1	Facial pain, vertigo, conv hysteria

Table II. *Bilateral hyperactive responses: Group B*

Diagnosis	No.	Symptoms
1 Central nervous system involvement	4	C. V A. with sudden left hearing loss (1) C. N. S. pathology Sp. nyst. Direct. prep. (1) Cerebral arteriosclerosis (2)
2. Basilar artery insufficiency	5	Vertigo, headache, normal hearing (4) Postural nystagmus (1)
3. Epilepsy	4	Vertigo (4) Normal hearing, spont. nystag. (1)
4 Head trauma	2	Skull fracture (1) Unilat. hearing loss (1)
5. Multiple sclerosis	1	Spont. nystagmus
6. Chronic alcoholism	1	Right hearing loss & tinnitus Right hemiparesis
7 Otosclerosis	2	
8. Psychosomatic disorder	6	Normal neurological findings Low fr. hearing loss and tinnitus (2) Presbycusis and vertigo (1) Vertigo (3)

creased from at least 42 beats for the first 25 sec of postrotatory nystagmus up to 59 beats in the same period in one case According to the neuro-otological, respectively neurological, diagnoses the list of these patients are shown on Table I.

Group B includes 24 patients as compiled in Table II.

In the psychosomatic category only one showed hypersensitivity following weak and strong stimuli whereas 5 patients exhibited increased responsiveness only after the weak stimulation, and none after the strong caloric effect.

Unilateral Hyperactive Response

As discussed above a strictly defined border line between normal and hyperactive is not possible. Therefore, except for extremely hyperactive responses there is no absolutely certain evidence whether abnormality is present. A hyperactive abnormality is more obvious when it appears on one side only Thirty-one such records were found. In addition to groups A and B it seemed to be appropriate to add the group C category (Figs 5 6) As a unilateral hyperactive manifestation this group with otherwise borderline values will become meaningful in contrast to normal average responses on the contralateral side

Group A consists of 7 patients as seen in Table III. Group B of 17 patients with unilaterally hyperactive nystagmus responses is shown in Table IV Group C of the unilateral hyperactive cases are presented in Table V

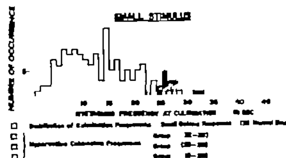


Fig 5 Unilateral hyperactive nystagmus response in 28 patients.

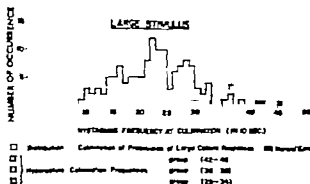


Fig 6 Unilateral hyperactive nystagmus response in 27 patients.

DISCUSSION

A distribution by diagnosis of all of our 68 patients with hyperactive vestibular responses clearly demonstrates the predominance of central pathology Arbitrarily we have classified "central nervous system involvement" separate from other more specific diagnoses such as basilar artery insufficiency multiple sclerosis, head trauma, etc. These are all, in fact, central involvements and it therefore becomes obvious and confirmatory that hyperactive nystagmic responses are caused most commonly by various central nervous system lesions In the relatively few cases where hyperactivity was found with a diagnosis of peripheral disease, such as otosclerosis or vestibular neuronitis, increased responsiveness was found mostly the contralateral side of the diseased labyrinth. Thus, such hyperactivity is not elicited primarily from the defective end organ, but may be a secondary central consequence of a unilateral peripheral lesion.

The "central nervous system involvement" is the largest diagnostic category Several of these cases had ataxia and other cerebellar symptoms and could often be determined neurologically as posterior fossa midline lesions as referred to by Riesco-McClure and others. Six out of the total of 19 in this category were labeled by neurology as severe cerebral arteriosclerosis or cerebrovascular pathology and 4 patients in this group had sudden total or subtotal deafness on one side Two of the patients had signs and history of recent encephalitis

Table III. *Unilateral hyperactive responses: Group A*

Diagnosis	No.	Symptoms	
1 Central nervous system involvement	2	Rt. hemisphere lesion with contralateral sudden hearing loss & tinnitus	(1)
		Postencephalitis ipsilateral deafness	(1)
2. Epilepsy	1	Vertigo, normal hearing	
3 Trauma	1	Lightning. Uncertainty and normal hearing	
4 Hereditary bilateral hearing loss	1		
5. Otosclerosis	2	Contralateral stapedectomy	(2)

Chronic intoxication of a Nembutal-Dexedrine addict is seemingly the cause of hyperactivity in one patient Rh incompatibility in another case In a few instances the neurological diag-

Table IV *Unilateral hyperactive responses: Group B*

Diagnosis	No.	Symptoms	
1 Central nervous system involvement	6	Bell's palsy contralateral vestibular areflexia, encephalitis?	(1)
		Contralateral sudden deafness, contralat. vestib. hyposensitivity	(1)
		Cerebrovascular lesion, presbycusis	(2)
		Cerebral atrophy contralat. hearing loss, perverted rotatory nystagmus	(1)
		Persistent imbalance, II spont nystag.	(1)
2. Epilepsy	1	(Jacksonian) craniotomy ataxia	
3. Trauma	1	Whiplash injury backaches	
4 Hemangiomata (head and neck)	1	Contralateral deafness	
5. Vestibular neuronitis	1	Contralateral	
6. Sensorineural hearing loss & tinnitus	4	Ipsilateral	(2)
		Contralateral hearing normal	(2)
7 Otosclerosis	3	Mixed hearing loss.	(2)
		Contralateral stapedectomy	(1)

Table V *Unilateral hyperactive responses. Group C*

Diagnosis	No.	Symptoms	
1 Central nervous system involvement	3	Ataxia, cerebro-vascular disease	(1)
		Bilat. hearing loss, RH incompetence	(1)
		Nembutal-benzedrine addiction	(1)
2. Head trauma	2	Skull fracture, bilateral hearing loss	(1)
		Nonfatigable postural vertigo	(1)
3. Cochlear hearing loss and tinnitus (Menière?)	1	Contralat. shunt operation	
4. Postural vertigo	1	Benign paroxysmal type	

nosis was less specific: cerebral atrophy CNS pathology and hemisphere lesion" are mentioned, to complete the range of diagnoses in this group.

Basilar artery syndrome of vertebro-basilar insufficiency have been long recognized as a common cause of momentary vertigo and anatomical interference of the vascular system could often be verified by angiography All 7 of our hyperactive vestibular reactions were bilateral when such a neurological diagnosis was made. The neuro-otological clinical manifestations were various forms of postural vertigo and nystagmus headaches and unaffected hearing, except for presbycusis in few instances.

It was not realized by us until the data of vestibular hyperactivity were collected that epilepsy or equivalent psychomotor seizures can exhibit exaggerated nystagmus responses. All 7 of our patients within this diagnosis had electroencephalographic abnormalities. In 5 the hyperreactivity was bilateral and in 2 it was unilateral. One of the unilateral hyperactive cases had a traumatic Jacksonian type of epilepsy and craniotomy was performed on the same side as the increased responsiveness. In all cases, hearing was normal Vertigo has been a component symptom either during the aura or of the attack of petit mal

Head trauma is a very common reason for long-lasting vertiginous symptoms The specific cause is unknown and commonly these complaints are not accompanied by vestibular deficit. It can apparently be associated with increased vestibular responsiveness as shown in 3 of our patients producing a bilateral hyperactivity and 4 others where the exaggerated nystagmic response occurred only on one side Most of these patients had postural nystagmus and vertigo which was either of the benign, fatigable variety or in one instance it was nonfatigable Two in this group suffered radiologically proven skull fractures, one had a whiplash injury and in one case the trauma resulted from lightning.

Abnormal vestibular findings are common in multiple sclerosis. Changing forms of spontaneous nystagmus and vestibular function deficit or hypoactivity are the more common abnormalities. That multiple sclerosis can cause hyperactive responsiveness as our 2 cases demonstrate, was unexpected. The hyperactivity was bilateral in both of our patients. The disseminated foci could perhaps affect nystagmus-inhibiting areas inasmuch as the demyelinization occurs directly along the nystagmus pathways in other instances.

In one of our patients with an ocular type spontaneous nystagmus, the increased vestibular nystagmus may be the result of an additional effect of an existing eye muscle incoordination problem with the vestibular reflex.

Acute alcohol intoxication is known to create mainly postural nystagmus and facilitates nystagmic activity generally Our single case of increased responsiveness in chronic alcoholism is certainly a central anomaly and could be based on such neuropathological conditions as are evidenced in acute intoxication.

It is logical to assume that diffuse and multiple hemangiomata on the head and neck have such extensions intracranially as to become responsible for hyperactive responsiveness as seen in one of our patients.

A school audiometric survey has discovered in a 10-year-old boy a 50 to 60 dB bilateral

hearing loss. Our supporting vestibular examination found a right-sided hyperactive response (type A) As usual, the central neuropathology is uncertain or unknown as regards the cause of such a hereditary (genetic) hearing defect. Neither is the question answerable as to the causes of unilateral vestibular hyperactivity No doubt the basic cause could be the same for the two entirely different manifestations

Another patient, a 14-year-old youngster acquired a unilateral total deafness with marked tinnitus but no symptoms of vertigo or dis equilibrium. The caloric testings revealed a marked (type A) hyperactivity on the same side *The cause could have been viral but it* was not specifically determined. In 4 patients with unilateral sensorineural hearing loss and tinnitus of unknown etiology an ipsilateral vestibular response increase was found in 3 patients while the fourth case exhibited a contralateral hyperactivity

These various neuro-otological clinical entities discussed so far include 51 patients where hyperactive vestibular responsiveness was manifest on both sides or only on one Seventeen patients in our material were diagnosed with terms signifying peripheral diseases or conditions. As many as 8 patients with the diagnosis otosclerosis were found to show increased vestibular responsiveness. In 3 the finding was bilateral and in 5 unilateral. The bilateral findings concerned advanced cases of otosclerosis with markedly mixed hearing loss in 3 elderly patients. In the 5 otosclerotic patients with unilateral hyperactivity 3 had stapedectomies on the contralateral side These were surprising findings which may lead to the speculation that either the stapes operation could be responsible for the contralateral hyperactivity or to the contrary the operation eliminated the increased nystagmic reaction on the operated side. In an independent vestibular study practically all stapes-operated labyrinths showed reduced vestibular sensitivity to a greater or lesser degree In cases of postoperative fistula, a reduction of sensitivity is more prominent. These observations tend to indicate the assumption that by reducing the response sensitivity on the side of operation we might have created a hyperactivity on the non-operated side in an individual who was relatively hyperactive on both sides before surgery All of our otosclerosis cases showed a mixed hearing loss and none of these 8 patients was younger than 51 years

One patient on the list appears with the diagnosis of Menière's disease. This may be misleading because this 42 year-old female developed a unilateral hearing loss with constant tinnitus without experiencing any dizziness. Her endolymphatic hydrops diagnosis was *entirely hypothetical and in the author's view* she had no Menière's disease. An endolymphatic shunt operation was performed with the hope of stopping the tinnitus This was not successful, and in addition a moderately hyperactive vestibular response was found on the same side.

Finally one patient was diagnosed as having vestibular neuronitis. The caloric nystagmus with both weak and strong stimulation was increased on the contralateral side, whereas on the side of involvement, a hypoactive response was obtained. An objective evaluation is difficult. By using our criteria, a type B hyper*activity was found and it is possible that prior* to the neuronitus attack this patient could have been type B on both sides. The culmination values with weak stimulation were 9 and 76 respectively right and left while the strong stimulation elicited a 12–38 culmination. The asymmetry is very marked indeed, but because in our normal population of 133 ears we have found one individual with a 29 culmination for the weak stimulus and in another 39 frequency for the strong caloric effect, our patient, prior to his neuronitis, had perhaps only an unusually high nystagmus frequency and could not be considered as hyperactive.

*In the bilateral hyperactivity group of pa*tients a relatively high number were found with complaints of dizziness, uncertainty confusion, weakness, etc., where except for the exaggerated caloric nystagmus no pathological find-

ings could be obtained. The hearing was normal and a neurological examination could not determine any objective abnormality One of these patients exhibited an exceptionally increased responsiveness (type A) and 6 others a somewhat moderate hyperactivity All these patients were overly anxious and concerned about their complaints. They had fears of serious or life-threatening consequences of their many sensations and difficulties. They already had consulted numerous doctors and hospitals and many of them had taken a variety of drugs and all kinds of medicine. The impression they often gave us was consistent with the term. neurotic. This group is marked in our classification of diagnosis as psychosomatic disorders No unilateral hyperactivity was found in patients fitting in this category

CONCLUSIONS

An increased nystagmic response following routine caloric or rotatory stimulation does not represent an increased vestibular excitability The receptors of the vestibular end organs are not affected, but the responsiveness is exaggerated due to some change in the nystagmus pathway system When vestibular stimulation evokes undue autonomic nervous responses with or without an increase of the elicited nystagmus, this cannot be regarded either as an increased sensitivity of the receptors but rather as a less inhibited responsiveness of the autonomic nervous system. Such neurophysiological and sensory organ considerations lead to the assumption that increased vestibular responsiveness represents no labyrinthine disease but some lesions in the central nervous system.

For a critical review and study of such clinical findings as vestibular hyperactivity" the criteria of the phenomena had to be established. Because of a wide range of responses in normal subjects, a precise identification of the "hyperactive" is difficult. Types or categories of hyperactivity were selected, therefore signifying responses which are definitely above the intensities we found in normals (type A) and reactions which are partly above the 90% confidence limit of responses in young adults without any disease (type B) When an increased responsiveness occurred on one side only it became reasonable to distinguish a third category (type C) of hyperactivity which is entirely within the 10% maximum responses found in normals

Sixty eight patients records were found fulfilling the above criteria of hyperactivity A variety of clinical diagnoses was found in these patients. In 44 (65%) a central nervous system pathology was obvious In 17 patients (25) the clinical diagnosis indicated a peripheral o inner ear pathology Seven patients (10) with a variety of symptoms and complaints show no evidence or organic disease and were label as psychosomatics. In the intracranial pathology group, distinct cerebellar involvement as found in a few but the majority included all sorts of acquired or hereditary (genetic) d cases of the central nervous system. It is not only the cerebellum or vestibulo-cerebellar pathway lesions which are responsible for increased nystagmic responsiveness. as demonstrated experimentally but hemispheral lesions like epilepsy cerebral atrophy and other condi tions, may also produce exaggerated vestibular responses. In the 17 patients with diagnoses of labyrinthine disease the hyperactive response was often contralateral. This may suggest a secondary central interaction as the possible cause of hyperactivity In some other instances of unilateral hyperactivity with peripheral diagnosis, the criteria of hyperactivity could have been faulty The smallest group of our patients exhibiting very active and intense stimulatory nystagmus had no other detectable organic disease. In this category the hyperactive nystagmic reaction may be one manifestation of a generalized and functional increased neurologic responsiveness.

Hyperactive vestibular responsiveness is a clinical entity It can be detected by standard stimulation and sensitive and reliable nystagmography It can corroborate an already established diagnosis or may suggest a hitherto un-

detected central nervous system pathology It may also occur in severely neurotic, psychosomatic patients. In cases of labyrinthine disease a contralateral hyperactivity suggests a possible secondary central interference which needs to be investigated both experimentally and clinically

ZUSAMMENFASSUNG

Die Auslösung eines hyperaktiven Nystagmus durch normale Reizung des Vestibularapparates ist hinlänglich bekannt. Bisher wurde jedoch angenommen, dass zentrale pathologische Veränderungen einem verlängerten postkalorischen und postrotatorischen Nystagmus zugrunde liegen. In letzter Zeit konnte jedoch experimentell bewiesen werden, dass hyperaktiver Nystagmus durch Läsionen des Kleinhirns hervorgerufen wird. Um den hyperaktiven Nystagmus als klinischen Befund verwerten zu können, müssen zunächst Kriterien für dieses Phänomen festgelegt werden. Zu diesem Zweck wurde an 67 normalen Versuchspersonen mit schwachen und starken kalorischen Reizen Nystagmus ausgelöst. Die Nystagmusfrequenz wurde in der Kulminationsperiode gemessen, wobei weit verstreute Reaktionen gefunden wurden. Unter Verwendung dieser Daten als Basis wurden dann 1700 Patienten in drei hyperaktiven Reaktionsgruppen klassifiziert. Gruppe A zeigte Nystagmusintensitäten, die niemals in der Gruppe normaler Versuchspersonen gefunden wurden. In Gruppe B konnte erhöhte Nystagmusaktivität in einem Ausmass nachgewiesen werden, da nur selten bei normalen Personen gefunden wird (3 in 133 normalen Ohren). Gruppe C bestand aus Patienten mit pathologisch starkem Nystagmus, obwohl 10% aller normalen Subjekte ebenfalls solch erhöhte Frequenzen zeigten. Unsere Patienten, die in dieses Grenzgebiet fallen, müssen daher als potentiell hyperaktiv angesehen werden. Im Falle von einseitigem hyperaktivem Nystagmus wird man diese Patienten umsomehr als pathologisch klassifizieren müssen. Die postrotatorischen Nystagmusreaktionen wurden ähnlich klassifiziert. Bei unseren 68 hyperaktiven Nystagmus-Patienten konnte eine Vielzahl von zentralen Abnormalitäten nachgewiesen werden. Bei peripheren Veränderungen, wie z. B. Otosklerose oder vestibuläre Neuronitis zeigten einige Patienten hyperaktive Reaktionen jedoch an der kontralateralen Seite. Bei einer kleinen Gruppe von Patienten wurden unter dem verstärkten kalorischen Nystagmus keine organischen Veränderungen festgestellt. Die Patienten dieser Gruppe waren nur ängstlich und besorgt wegen Schwindelgefühl und Unsicherheit. Bei 7 von den 68 Patienten, die beiderseitige Hyperaktivität aufwiesen, wurde dies als psychosomatische Störung bezeichnet. Vorliegende Studien führen zu dem Schluss, dass hyperaktive Nystagmusreaktionen ein klinisches Bild darstellen, das durch standardisierte Labyrinthreizung und verlässliche Nystagmographie nachgewiesen werden kann. Dieses Phenomen konnte in einer grossen Zahl von Störungen des zentralen Nervensystems und gelegentlich in neurotischen Patienten festgestellt werden.

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EFFECT OF HEMICEREBELLECTOMY UPON THE CYTOCHEMISTRY OF NEURONS IN THE LATERAL VESTIBULAR NUCLEUS

II. *Effects on RNA Content and Succinoxidase Activity in Deiters Neurons after Warm and Cold Water Irrigation*

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Abstract. Giant nerve cells from the lateral vestibular nuclei in rabbits subjected to hemicerebellectomy and caloric irrigation were analyzed for their ribonucleic acid (RNA) content and succinoxidase activity The animals were irrigated with cold or warm water while still in anesthesia after hemicerebellectomy No statistically significant side differences were detected regardless of the side irrigated or the temperature used. The results are compared t the findings obtained in animals subjected to cerebellectomy and calorization, and to rabbits subjected to hemicerebellectomy only

The functional influence of unilateral caloric stimulation on cytochemical parameters in the Deiters giant nerve cells of the lateral vestibu lar nucleus and in the cerebellar Purkinje cells has been studied previously (Blomstrand et al 1966) It was shown that repeated unilateral irrigations with warm water in the outer ear of intact rabbits produced significant increase in the succinoxidase activity of the Deiters cells on the irrigated side while cold water resulted in a similar biochemical response of the contralateral Deiters cells. A single ir rigation did not result in any observable biochemical changes in the Deiters cells. After cerebellectomy however a single irrigation with warm or cold water gave the same suc cinoxidase changes as those described after r peated calorizations of intact animals At the same time the RNA content of the De e cells showed changes opposite to those o t succinoxidase activity (Blomstrand et al 1968) Both single and repeated irrigati ns af fected the RNA content of th Purkinje cells in the lobulus centralis and the nodulus with higher values ipsilaterally compared t the contralateral side after warm water irr gation and higher contralateral values after colo water (Blomstrand et al., 1966 Jarlstedt 1966) Considering the inhibitory action of the Purkinje cells on the Deiters cells (Ito & Yoshida, 1964) these data suggested an increased inhibitory effect on the Deiters cells counteracting the stimulatory effect on these cells caused by ipsilateral warm water calorization.

In part I of this study (Blomstrand et al., 1970) we found asymmetrical biochemical changes in the Deiters cells after removal of the right half of the cerebellum. In the pres ent work the influence of unilateral calorization on this changed balance within the vestibular system was investigated.

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MATERIAL AND METHODS

White rabbits of both sexes weighing 1.5–1.8 kg were used.

Table I *RNA content in Deiters giant nerve cells from hemicerebellectomLed rabbits (right sided ablation) after warm or cold water irrigation for 30 min*

Enzyme activities expressed as 10^{-4} µl O_2 per hour Mean value ±S.E.M

		Left side		Right side (operated)	
Type of irrigation	No of animals	No. of cells	Mean value ±S.E.M	No. of cells	Mean value ±S.E.M
( ) *Calorization on right (operated) side*					
48°C	6	47	1 155±82	44	1 197± 86
20°C	4	34	1 150±90	35	1 078±110
(b) *Calorization on left (nonoperated) side*					
48°C	4	31	1 206±97	33	1 076± 87
20°C	4	27	1 160±14	26	1 19± 44

No side d'fferences are statistically significant.

Hemicerebellectomy and caloric stimulation

The animals were anesthetized by intravenous injection of Mebumal (pentobarbital) sodium 6% (74 mg/kg) The right half of the cerebellum was extirpated as described in part I (Blomstrand et al., 1970) The caloric stimulation was performed immediately after the hemicerebellectomy Two series of animals were subjected to cold water (70 C) irrigation, in the left and right outer ear respectively for 30 min and the other two groups were treated similarly with warm water (48 C)

RNA content and succinoxidase activity

assays were made on single Deiters nerve ls as reported in part I (Blomstrand et al 970)

RESULTS

Animals

Postoperatively the animals showed a tendency of dorsal flexion of the neck and rotation to the left. A nystagmus adequate to the caloric stimulation was achieved about 30 sec after start of the irrigation, i.e nystagmus to the contralateral side using cold water and to the ipsilateral side using warm water

RNA

The results are presented in Table I The RNA content of the Deiters cells did not show any statistically significant side differences, but there were reproducibly higher ipsilateral RNA contents using warm water while there were reproducible slightly higher contralateral values after cold water irrigation. These changes appeared regardless of whether the irrigation was performed on the side of hemicerebellectomy or on the side with intact cerebellum. The RNA content of the Deiters cells was also slightly lower in all calorized groups as compared to animals subjected solely to hemicerebellectomy and killed after 1 hour (Blomstrand et al., 1970)

Succinoxidase activity

The results are presented in Table II. The succinoxidase activity did not show any statistically significant side differences. In the rabbits calorized with warm water on the left side, i.e the side contralateral to the cerebellar ablation, the Deiters cells had a succinoxidase activity somewhat higher on the irrigated side.

DISCUSSION

In our earlier investigations the important role of the cerebellum as a regulator and integrator of the vestibular system was stated (Blomstrand et al., 1966 1968 Ito & Yoshida, 1964) However contralateral biochemical effects in the Deiters cells could be elicited after

Table II. *Succinoxidase activity in Deiters giant nerve cells from hemicerebellectomized rabbits (right sided ablation) after warm or cold water irrigation for 30 min*

Enzyme activities expressed as 10^{-4} μl O per hour Mean value ±S.E.M.

	Left side			Right side (operated)	
Type of irrigation	No. of animals	No. of cells	Mean value ±S.E.M	No. of cells	Mean value ±S.E.M
(a) Calorization on right (operated) side					
48°C	4	8	4.4±0.9	7	6.0±1.7
20°C	4	7	4.7±0.7	9	4.9±0.9
(b) Calorization on left (nonoperated) side					
48°C	5	7	7.6±1.4	9	5.0±1.0
20°C	4	6	5.4±1.0	11	5.9±0.4

No side differences are statistically significant.

unilateral caloric stimulation in cerebellectomized animals (Blomstrand et al., 1968). This contralateral effect was decreased if the animals were subjected to cerebellectomy and a midline section of the medulla oblongata from the colliculi down to obex (Blomstrand et al., 1970) and a similar brain stem division has been shown to eliminate inhibition of the contralateral vestibular activity otherwise evoked by electrical stimulation of one of the vestibular nerves (Shimazu & Precht, 1966).

On the basis of the bilateral increase in extent of the Deiters cell biochemical responses to calorical stimulations occurring in animals totally deprived of the inhibitory influence of the cerebellum (Blomstrand et al., 1968) cytochemical changes would be expected on the side of hemicerebellectomy after ipsilateral warm water or contralateral cold water irrigation. Our present results, however did not show any marked side differences, neither after irrigation on the side deprived of most of the cerebellovestibular inhibition nor after irrigation on the intact side. Only after warm water irrigation on the side contralateral to the hemicerebellectomy was a slightly higher succinoxidase activity obtained on the irrigated side. The exact reason why no greater cytochemical side differences were observed using irrigation of the different sides in the hemicerebellectomized animals is not yet known. However cytochemical differences between vestibular nerve cells from the right and left side are probably related to functional imbalances within the vestibular system and the failure of any clear biochemical side differences after unilateral calorizations in hemicerebellectomized animals may reflect the action of compensating mechanisms within the vestibular system.

Thus, there are other areas than the cerebellum, important for the cytochemical balance within the vestibular system such as peripheral spinal, subcortical and cortical structures. Yules et al. (1966) observed that the nystagmus elicited by unilateral stimulation of the lateral vestibular nucleus could be both inhibited and facilitated by stimulation of either side of the mesencephalic reticular formation. According to their study there must exist both inhibitory and facilitatory pathways, originating from the reticular formation, crossing the midline. The superior cerebellar peduncle decussations were also found in the reticular formation area and its fibers might be closely connected to the reticular formation inhibitory paths, since contralateral inhibition responses were common here.

From the present data it is evident that the remaining half of the cerebellum together with subcortical, bulbar and spinal or peripheral structures is able to compensate for unilateral influences and thus inhibit biochemical imbalances between the Deiters nerve cells of the different sides.

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ZUSAMMENFASSUNG

Riesennervenzellen vom lateralen vestibularen Kern von Kaninchen behandelt mit Hemicerebellectomie und Wärmespülung wurden auf ihren Ribonucleinsäuregehalt (RNA) und Succinooxydaseaktivität hin untersucht. Die Tiere wurden noch unter Betäubung nach Hemicerebellectomie mit kaltem oder warmem Wasser gespült. Keine statistisch bedeutenden Seitendifferenzen wurden gefunden ohne Rücksicht darauf welche Seite gespült wurde oder welche Temperatur zur Anwendung kam. Die Resultate sind mit den erhaltenen Befunden von Tieren, die Cerebellectomie und Kalorisation unterworfen wurden, mit den Befunden von Tieren, die nur cerebellectomiert wurden, verglichen worden.

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TOMOGRAPHIC EXAMINATION OF CHOLESTEATOMAS IN THE MIDDLE EAR

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Abstract. A retrospective investigation of the diagnostic certainty in radiological examinations of cholesteatomas in the middle ear has been made. Through period of six years the security has proved to be more than 90%. Small cholesteatomas which have not yet caused osseous destruction and cholesteatomas in earlier operated ears are barely accessible to radiologic evaluation. The oto-radiological experience of the describing radiologist must be emphasized.

In spite of adequate therapy complications in chronic otitis media do still occur—here especially referring to cholesteatomas.

Clinical examinations are likely to provide information of an actual cholesteatoma in about 60–70% of cases, but sometimes it may prove necessary to supplement clinical examination with roentgenological in order to state the diagnosis more precisely A watchful attitude and conservative therapy is permissible in chronic otitis media, whereas the presence of a cholesteatoma indicates surgery to prevent the complications which might otherwise occur—such as destruction of the ossicles, the labyrinth with a labyrinthine fistula or destruction of the facial canal, or even destruction of the tympanic tegmen with secondary symptoms of meningitis or encephalitis.

Conventional radiologic examination of the temporal bone will reveal cholesteatoma in 60–70% of cases It has been proved that with tomography it is possible to diagnose a cholesteatoma with up to 94% certainty as has been verified by Brünner et al. (1966) in those ch esteatoma cases operated.

The investigations in question took plac. from 1958–62. To evaluate whether diagno i certainty has increased, data of radio ogica e c aminations from all patients with verified chol esteatomas from 1963–68 have been reviewed retrospectively and the tomographic findings compared with the operative

MATERIAL

In the years 1963–68 52 patients with operatively verified cholesteatomas have been tomographed in Gentofto Hospital, Copenhagen. Twenty-nine of the patients were males, and 23 females. Thirteen patients were less than 20 years old, and of these, 6 were less than 10 years old 8 patients were more than 60 years old.

Radiological Examinations

Various tomographic projections have been suggested in infectious conditions of the temporal bone In our experience the following two projections must be recommended. The *A p* (frontal) projection, and the lateral (Brünner 1969) A frontal tomography using a Massiot Philips Polytome was carried out on all the patients. In some cases—especially when complications like a facial palsy were present—

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THE EFFECT OF SEMICIRCULAR CANAL STIMULATION DURING TILTING ON THE SUBSEQUENT PERCEPTION OF THE VISUAL VERTICAL

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(Received March 31 1970)

Abstract When a man is accelerated on a centrifuge, the direction of gravitoinertial vertical changes relative to his body However a lag occurs in his perception of this change. The hypothesis has been advanced that the perceptual lag in this situation is partly the result of a conflict between signals arising from the semicircular canals and from the otolith organs. To test this hypothesis, subjects were tilted in such a way that they received consistent semicircular canal and otolith signals. This was accomplished simply by tilting them 30 deg from upright in their frontal plane. Immediately after being tilted, these subjects made estimates of the vertical which were approximately accurate and they continued to make accurate estimates throughout a 140 sec judgment period. The absence of a perceptual lag under these circumstances supports the hypothesis.

In flight, as in natural movement, the semicircular canals and otolith structures are usually stimulated simultaneously Typically these receptors have been studied in the laboratory as if they were independent systems, but the importance of considering interactions between them has been indicated in the past (Löwenstein, 1956 Tait & McNally 1934) and has been re-emphasized by recent evidence. Several studies suggest that semicircular canal responses exert an influence on the dynamic perception of the vertical" (Correia & Guedry 1966 Guedry 1965 1966) In those experiments, the subject was positioned so that his ~axis was Earth-horizontal, and then he was rotated about that axis This type of rotation produced continuous reorientation of the otolith organs with respect to gravity At 30 rpm, subjects were approximately correct in signalling orientation relative to gravity for a period of 30 to 50 sec, that is, during the period of time required for the cupula response to subside thereafter they were unable to signal their orientation correctly (Correia & Guedry 1966). When the speed of rotation was reduced to 10 rpm, most subjects perceived their orientation throughout the period of rotation. From these results, Guedry (1966) concluded that during a rapid change in the direction of a linear acceleration vector the direction of the vector is not perceived accurately when otolith information is not supplemented by appropriate information from the semicircular canals.

A similar interpretation was applied to results from a quite different experimental situation in which subjects were exposed to linear oscillation on a parallel swing (Guedry & Harris, 1963 Schöne & Mortag, 1968) and on a horizontal track (Niven et al., 1966) When subjects were firmly restrained during oscillation on either of these devices, they were subjected to no angular acceleration therefore, canal stimulation was nil (according to classi-

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Opinions or conclusions contained in this report are those of the uthors. They are not to be construed as necessarily reflecting the view or the endorsement of the Departments of the Army or Navy

cal concepts), whereas the resultant linear acceleration vector oscillated back and forth through angular displacements as large as 30 deg. Subjects in this situation felt only slightly tilted (Schöne & Mortag, 1968) or experienced no tilt at all (Guedry & Harris, 1963 Niven et al., 1966) implying again that, when supplementary canal information is lacking, perception of tilt is minimized.

Guedry (1966) suggested that a similar process may be in part responsible for the lag in the perception of gravitoinertial vertical (Clark & Graybiel, 1966 Graybiel, 1952) because when a subject is seated upright at a distance from the center of rotation on a centrifuge the initial angular acceleration causes the semicircular canals to signal rotation in a plane that is perpendicular to the one in which the gravitoinertial force changes direction. Thus, in this case, the canals not only fail to provide supplementary information regarding the axis of tilt, but they produce signals that conflict with those coming from the otolith organs. The lag in perception of the gravitoinertial vertical on the centrifuge must be largely the result of the manner in which the subject is tilted and probably is due to discordance between canal and otolith information during the initial period of tilt.

If, instead, a subject were to be tilted in the normal manner that is, simply tilted with respect to gravity about an Earth-horizontal axis with an acceleration profile to permit supplementary canal information regarding angular displacement (Owens & Guedry 1969), then perception of bodily orientation should be both immediate and veridical. The present experiment was undertaken to test this prediction.

PROCEDURE

Subjects

Eighteen young men with no apparent vestibular defects served as subjects for this experiment. Nine of the subjects were laboratory personnel who had previous experience on the device the remaining were student aviators.

Apparatus

The rotary device used in this experiment has been described previously (Guedry 1965) Briefly the device, as used in this experimen allowed the subject to be tilted in the y (frontal) plane[1] either clockwise or counter clockwise to a predetermined tilt position ( ) with independently variable acceleration (6 deg/sec² maximum) and velocity (18 deg sec maximum) The experimenter was able t monitor continuously the inclination of the subject within 0.5 deg. Subjects were firmly secured by means of safety straps across the head, chest, thighs, and feet to a metal chair mounted on the tilt device

An illuminated column of light, 1 mm b 10 cm, was mounted in front of the subject at a distance of 1 m. The lighted column cou d be rotated either clockwise or counterclockwise m the subject s y z plane by the experimen or by the subject (by the latter with hand-h pushbuttons) The experimenter could read th inclination of the lighted column to the nearest 0 1 deg. The control and readout mechanism for the lighted column has been described by Correla et al. (1965)

The entire apparatus was draped with opaque material to exclude all extraneous light.

Method

The tilt angle, ϕ_x, used in this experiment was 30 deg. One group of 6 subjects was tilted at a rate well above the threshold for canal stimulation. The angular acceleration, a_x, for these subjects was 6 deg/sec² followed by a 6 deg/sec² deceleration. The other group of 6 subjects was tilted slowly at a constant velocity of 1 deg/sec The small angular impulses involved in starting and stopping rotation at 1 deg/sec are near absolute threshold for canal stimulation, so this group did not receive canal information which by itself would permit accurate perception of angular displacement (Owens & Guedry 1969)

[1] The nomenclature system proposed by Hixson et al. (1966) is used here.

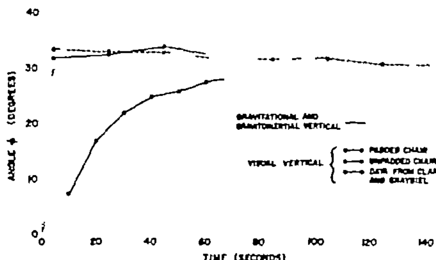


Fig 1 Mean estimates of visual vertical for subjects tilted at the rate of 6 deg sec² Data were combined for both right and left directions of tilt. On the ordinate angular displacements are e pressed in degrees from the subject s -axis. The solid line represents approximate angular displacement from the subject s z-axis of the gravitational vertical in the present experiment and of the gravitoinertial vertical in Clark & Graybiel (1966) Plotted circles (open, closed, or dotted) represent angular displacement from the subject -axis of the lighted column used to indicate the visual vertical.

The accuracy of estimates made by these two groups of subjects suggested the possibility that somesthetic cues were a more important factor in the present experiment than in the situation used by Clark & Graybiel (1966) Since it was desired to compare the present results with those of Clark & Graybiel, the entire experiment was repeated with the chair well padded and the subjects wearing an orthopedic neck brace to minimize head movements Six ubjects were tilted quickly and 6 subjects were 'lted slowly under this condition. Some of these subjects were the same as used previously

The experimental procedure the same for all subjects regardless of the group to which they were assigned, was as follows The subject was secured in the chair and positioned so that his axis was aligned with gravity He was instructed to keep his eyes closed at all times except when making a judgment. Then the experimenter offset the lighted column and instructed the subject to align it with Earth-vertical This procedure was repeated three more times The mean of these four judgments was defined as the visual vertical and was used as a reference in calculating accuracy of all subsequent judgments The subject was then tilted 30 deg to his left side and immediately instructed to align the lighted column with Earth-vertical When he completed his judgment, he signalled the experimenter and closed his eyes whereupon the experimenter offset the line for the next trial. (Magnitude and direction of the offset occurred in a quasirandom sequence) This procedure was repeated at 20 sec intervals until the subject had made eight judgments. Then he was rotated until he achieved a 30 deg tilt to his right side and the procedure was repeated. In total, each subject made eight judgments at each of six successive 30 deg tilts, three to his left side and three to his right, in alternating sequence

RESULTS AND DISCUSSION

Under all conditions of the experiment, subjects were able to estimate accurately the visual vertical immediately upon being tilted and continued to make accurate estimates throughout the judgment period Subjects who received deg/sec² angular acceleration while being tilted (Fig. 1) appeared to show an Aubert effect whereas subjects tilted at 1 deg/sec² (Fig. 2 displayed a slight Muller effect. The difference

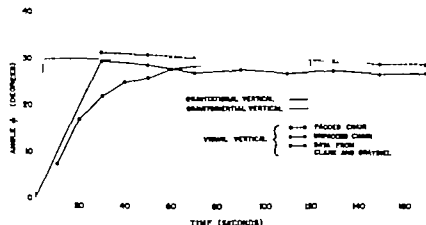


Fig 2 Mean estimates of visual vertical for subjects tilted at rate of 1 deg/sec. Data were combined for both right and left directions of tilt. On the ordinate, displacements are expressed in degrees from the subject z-axis. The solid line represents approximate angula displacement of gravitational vertical from the subject's z-axis in the present experiment. The dotted line represents approximate al displacement f the gra itoinertial ertical from ject z-axis in Clark & G ybiel (1966) Plo circles (open, closed, or dotted) represent an displacement from subject' z-axis f the lighted I umn used to indicate the visual vertical

between judgments made under these two conditions was significant at $p = 0.05$ but did not reach significance at $p = 0.01$ Thus it appears that the faster tilt caused subjects to displace visual vertical farther from their own -axis, but this effect was a weak one, amounting to an average of about 3 7 deg.

For subjects who received a 6 deg/sec² angular acceleration while being tilted, stimulation of the semicircular canals provided information about the axis of tilt that supplemented information received from the otolith organs. Visual vertical estimates by these subjects can be compared in Fig. 1 with estimates of the visual vertical made by subjects who rapidly underwent a 30 deg change in the direction of the gravitoinertial force on a centrifuge (Clark & Graybiel, 1966) The latter subjects displayed the familiar lag effect.

Fig. 2 shows the estimates of groups which were tilted at a rate near the threshold of canal stimulation. It appears from these data that the lack of adequate canal information does not in itself impair the accuracy of estimating verticality However in this case the tilt was accomplished very slowly over 30 sec were required to achieve the full 30 deg tilt. In previous studies, when subjects wer oscillat on a parallel swing (Guedry & Harris, 1963 Schöne & Mortag, 1968) or on a horizonta track (Niven et al., 1966) the resultant linear force changed direction relative to the body more quickly and little or no tilt was perceived. In other words, in the absence of supplementary canal information, the more rapid tilts were not perceived. Thus it appears that subjects are able to accurately estimate visual vertical soon after being tilted without supplementary canal information, but only when the tilt is sufficiently slow

A comparison is made in Fig. 2 between estimates of visual vertical following slow tilt in the present study and those made by subjects tilted relative to the gravitoinertial vertical on a centrifuge (Clark & Graybiel, 1966) It can be seen that, whereas subjects in the present study made accurate estimates of visual vertical immediately after achieving full tilt, subjects on the centrifuge made estimates that amounted to only about 80% of the true change in gravitoinertial vertical after 30 sec of constant rotation This difference, though not large may indicate that discordant canal information retards the accurate perception of

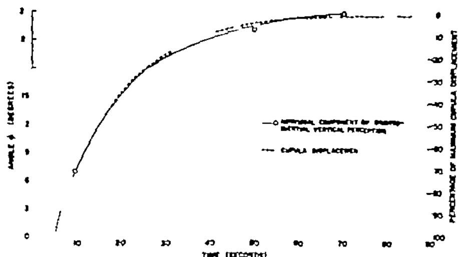


Fig 3 Comparison of theoretical values of cupula displacement with the nonvisual component of gravitoinertial vertical perception reported by Clark & Graybiel (1966). Cupula displacement derived according to van Egmond et al. (1949) assuming a time constant of 15 sec.

the vertical more than does the absence of a canal input.

Graybiel and his associates have thoroughly investigated the perception of gravitoinertial vertical they succeeded in separating two interacting components of the effect, a visual and a nonvisual one Clark & Graybiel (1966) suggested that the interaction of the visual and nonvisual factors is responsible for the lag in perception of gravitoinertial vertical To explain the nature of the interaction, they used Helson's concept of "cross modality interaction," according to which incoming information from the various sense organs is weighted form immediate percepts. They assumed that visual factors, which inform the subject that he is aligned with gravity receive less weight as rotation continues, while nonvisual factors, telling him that he is tilted, receive increasingly greater weight. The shift in weighting between these two factors is presumed to lag behind the change in direction of the resultant force, so a lag appears in perception of the gravitoinertial vertical.

The results of the present study complement those of Clark & Graybiel (1966) by suggesting a reason why the nonvisual factor should receive increased weight during constant velocity rotation. At the beginning of rotation, semicircular canals produce discordant information which impairs perception of the direction of the gravitoinertial force The canals signal rotation about the subject's z-axis, whereas the change in direction of the gravitoinertial force implies rotation primarily about the subject's x-axis After constant velocity is reached, canal response slowly subsides, the conflict diminishes, and veridical perception is attained.

Support for this interpretation comes from the striking similarity between the rise in the nonvisual component and the decay of semicircular canal stimulation, as shown in Fig. 3. The solid line represents the nonvisual component of the perception of the gravitoinertial vertical, described by Clark & Graybiel (1966). The dotted line represents the theoretical function for cupula return following an angular acceleration, assuming a time constant of 15 sec (van Egmond et al., 1949) The close relationship shown here is what one would expect if discordant canal signals contribute to the lag in perception of the gravitoinertial vertical on the centrifuge

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ZUSAMMENFASSUNG

Wenn jemand in einer Zentrifuge beschleunigt wird, ändert sich die Richtung der gravitoinertialen Senkrechten relativ zu seinem Körper Es tritt aber eine Verzögerung in seiner Wahrnehmung dieser Ver-

änderung ein. Es ist die Hypothese vorgeschlagen worden, dass die Wahrnehmungsverzögerung in dieser Situation zum Teil die Folge eines Konfliktes zwischen in den Bogengängen und den Otolithen entstehenden Signalen ist. Um diese Hypothese zu testen, wurden Versuchspersonen in einer Weise geneigt, dass sie übereinstimmende Bogengang- und Otolithsignale empfingen. Es wurde dies einfach so bewerkstelligt, dass sie um 30° in ihrer frontalen Ebene aus der Senkrechten geneigt wurden. Unmittelbar nach der Neigung machten diese Versuchspersonen Schätzungen der Senkrechten, die angenähert richtig waren, und verharrten bei richtigen Schätzungen über eine 140 Sekunden Urteilsperiode. Das Fehlen einer Wahrnehmungsverzögerung unter diesen Umständen unterstützt die Hypothese.

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HUMAN TYMPANIC MEMBRANE

An Ultrastructural Observation

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Abstract Submicroscopic details of the human tympanic membrane were studied utilizing a transmission electron microscope (T E M) and a scanning electron microscope (S. E. M.) Three layers were distinctly recognized outer epidermal, middle lamina propria and inner mucous layer In pars flaccida, the middle lamina propria is formed of loose connective tissues with abundant elastic and collagen fibers. On the other hand, in the pars tensa, the middle lamina propria is formed of outer radial and inner circular fibers. Besides these two types of fibers, parabolic fibers were observed between the radial and circular fibers. The fibrils of the pars tensa fibers are rectangular in cross-sectional iew and are mixed with varying amounts of collagen fibrils. As in animals, the human pars flaccida was considerably thicker than the pars tensa, contrary to the popular concept t they are thinner

) earlier account of an electron microscopic observation on the human tympanic membrane was *reported by Lim (1968 a b) who demonstrated* that the pars flaccida was considerably thicker than the pars tensa in laboratory animals, and also by Johnson et al (1968) It was also reported by the above authors that the fibers of the *lamina propria of pars tensa* are formed by rectangular fibrils, in a cross-sectional view which are much finer than collagen fibrils. Recently Hentzer (1969) investigated the human tympanic membrane with an electron *microscope and concluded* that the fibers of the lamina propria of humans are collagen.

Nishiyama (1937) was the first to report the existence of elastic fibers in the human tympanic membrane, although Filogamo (1949) did not find them.

The present investigation was undertaken to establish the ultrastructural morphology of the human tympanic membrane to serve as a base line in interpreting middle ear pathology such as chronic otitis media and acquired cholesteatoma.

METHOD

Twenty normal adult tympanic membranes were obtained from autopsies. Two to five *hours post mortem, the temporal bones* were removed with a ring saw and fixed immediately in cold buffered 2% glutaraldehyde solution. Fixation time varied from 6 to 24 hours. Following the initial fixation, the temporal bones were washed *with Ringer solution* and the tympanic membrane was dissected in toto under a surgical microscope. After dissection, the tympanic membrane was post fixed in cold 1% osmic acid for 1 to 2 hours and dehydration was *carried out in graded* alcohol. The entire tympanic membrane was then embedded in epon and the desired areas were remounted for thick sections (2–3 μm) for light micro-

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scope and thin sections for electron microscopy utilizing an ultratome (LKB) The thin sections were double-stained and examined with an electron microscope (Philips 300) with magnification ranging between 600 and 50 000

For scanning electron microscopic examination, the middle fiber layer was exposed by removing the epidermal layer during dissection, and coated with gold (200–300 A) in a vacuum evaporator on a rotating stage The specimens were examined with a scanning electron microscope (Cambridge Stereoscan) with magnification ranging from 20 to 20 000 at an accelerating voltage of 10 kV

Gross Anatomy

The shape of the human tympanic membrane is conical, the apex of which forms an umbo Pars tensa represents the major area of the cone. Pars flaccida on the other hand, is identified as a small inverted triangle above the short process of the malleus The annular ligament is distinctly recognized surrounding only the pars tensa but is lacking at the margin of pars flaccida which is a continuation of the external auditory meatus Therefore, no clear distinction between the external auditory meatal skin and pars flaccida can be made The longest diameter of the tympanic membranes measures between 9.0 mm and 10.2 mm while the shortest diameter varies between 8.5 mm and 9.0 mm. After removal of the epidermis, the radial and circular fibers can be distinguished. Those fiber layers were formed of bundles of fibers.

Light Microscopic Observation

The thickness of pars flaccida measures between 0.03 and 0.23 mm, whereas pars tensa measures 0.03 mm and 0.09 mm. The pars flaccida in humans, like in lower animals, has a lamina propria of considerable thickness of fairly loose connective tissue with abundant elastic fibers (Fig. 1 *A*) Myelinated as well as unmyelinated nerve fibers and blood vessels are readily found in this layer The epidermis is composed of five to ten cell layers of desquamating epithelial cells, whereas the mucosal cells are, in general, of the simple squamous type except near the transition between the lining of the middle ear cavity where they become stratified or a simple columnar type Occasionally some of the mucosal cells possess cilia.

The fiber arrangement of pars tensa is distinctly recognizable at the light microscopic level with well-developed outer radial fibers and moderately well-developed inner circular fibers (Fig. 1 *C*) The fibers in the pars tensa gradually thin out as they reach the pars flaccida (Fig. 1 *B*) Our measurement with the light microscope suggests that the pars tensa is thinner than that of pars flaccida and that the lamina propria of the latter is composed of loose connective tissue lacking the well-developed fiber system present in pars tensa.

Electron Microscopic Observation

A. *Pars flaccida*

The epidermis of the pars flaccida is composed of five to ten cell layers of desquamating epithelial cells The epidermal cells are identical with that of animals reported earlier The epidermis is composed of a basal cell layer a Malpighian layer a granular cell layer and a keratin layer The basal cells are somewhat cuboidal. The basal cells contain abundant keratin fibrils, and well-developed mitochondria in their cytoplasm. The base of these cells is delineated by a well-developed basement membrane Small round or oval bodies with lamellated structures begin to appear in Malpighian cell layer near the granular cells. These bodies, called membrane coating granules or Odland bodies, are much smaller than the mitochondria and measure about 0.2 μ in diameter They are extruded from the cell in the granular layer and become amorphous and extremely flat in the keratin layer Finally they are found between the stacks of keratin plates.

The lamina propria of the pars flaccida is composed of abundant irregularly-arranged col

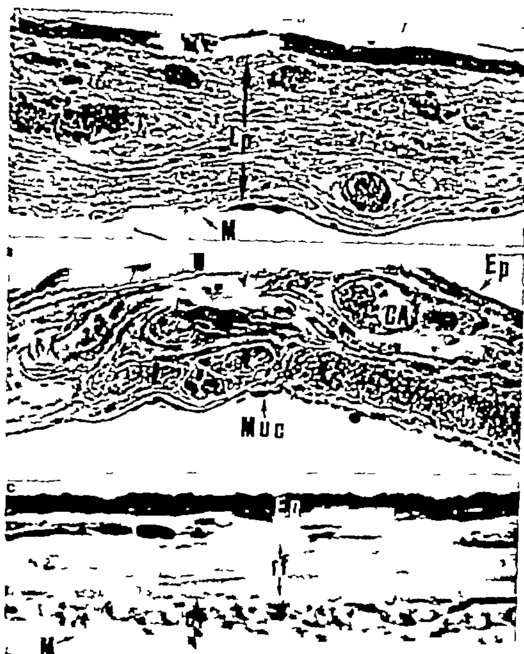


Fig 1 (*A*) Phase contrast micrograph of Shrapnell' membrane illustrating from top, epidermis (*Ep*), lamina propria (*Lp*) and mucosal layer (*M*). Lamina propria is formed f loose connective tissue Numerous blood vessels and nerves were found. Some of the mucosal cells are ciliated. 280 (*B*) Transition between pars flaccida on the left, and pars tensa on the right. Observe the fiber arrangement (*f*) which is interpreted as a cross section of circular fibers which gradually become smaller and eventually are no longer traceable in the pars flaccida proper (*Ep*), epidermis, (*N*), nerve fiber (*CA*), capillary / circular fibers; (*Muc*), mucosa. 280 (*C*) A phase contrast micrograph of pars tensa illustrating epidermis (*Ep*), radial fibers (*rf*), circular fibers (*cf*) and mucosal layer (*M*). Observe the subepidermal layer which is formed of loose connective tissue between epidermis and compact fiber layers. 570



Fig. 2. (*A*) High power view of collagen fibrils from pars tensa demonstrating typical collagen banding (640 Å). (*B*) Longitudinal section of fine fibrils from the pars tensa. No banding is observed. (*C*) Cross-sectional view of radial fiber which is formed from finer and larger fibrils. Large round ones are collagen, but the finer ones are rectangular suggesting they may be composed of four sub-units. (*D*) A cross-cut view of elastic (*El*) and collagen (*C*) fibrils show numerous dark cores in homogenous ground substance. (*E*) Longitudinal section of elastic fibrils (*El*) showing no banding. Randomly arranged collagen (*C*) fibrils can also be seen. A fibrocyte-like cell with abundant cytoplasmic tonofilaments is shown.



Fig 3 (*A*) Mucosal cells (*M*) of Shrapnell's membrane are shown. These cells possess well-developed microvilli on the free surface and pinocytotic vesicles at the basement membrane side. A collapsed capillary (*CA*) is shown in the lamina propria. Elastic fibers (*l*) are intermixed with collagen fibers in this layer (*B*) Mucosal epithelium of Shrapnell's membrane showing few cells with light granules (*arrow*) which resemble the secretory droplets of the goblet cell. (*SC*) secretory cell (*BM*), basement membrane.



Fig. 4. A transmission electron micrograph illustrating the entire thickness of the pars tensa. From the top: epidermis (*Ep*), subepidermal connective tissue layer (*Derm*), radial fibers (*Rf*), circular fibers (*Cf*) and mucosa (*M*). Between the fiber bundles, cells resembling fibrocytes are seen. Lamina propria in pars tensa constitutes dermis, radial and circular fiber layers. In some areas of pars tensa there is thin layer of connective tissue between circular fibers and mucosal layer which is not evident in this micrograph.

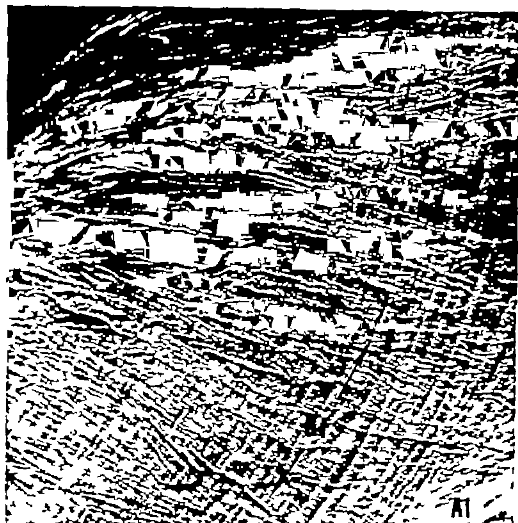


Fig. 5. A scanning electron micrograph illustrating ...al fiber arrangement of the tympanic membrane ...e anterior-inferior quadrant. The epidermis layer *was removed after fixation to expose the fiber layers.* (*Um*), umbo (*Rf*), radial fibers; (*Cf*), circular fibers; (*Al*), annular ligament.

lagen and elastic fibers. Large numbers of capillaries and nerve fibers also were found in this layer The nerve fibers were both myelinated and unmyelinated However specialized nerve endings were not found. In this layer the large numbers of fibroblasts were obser... Occasionally a cell resembling a fibroblast ...peared near the elastic fibers. This cell ...tained numerous tonofilaments in its ... and are interpreted as elastoblasts (Fig. 2 ... Collagen fibrils in pars flaccida show ... collagen banding of 650 A (Fig. 2 *A*) ... the somewhat amorphous elastofibrils show ... banding except streaky dark filaments ... bedded in an amorphous background (Fi...

2 *E*) In a cross-cut section, the elastofibrils show ... k cores among the amorphous ... (Fig. 2 ...

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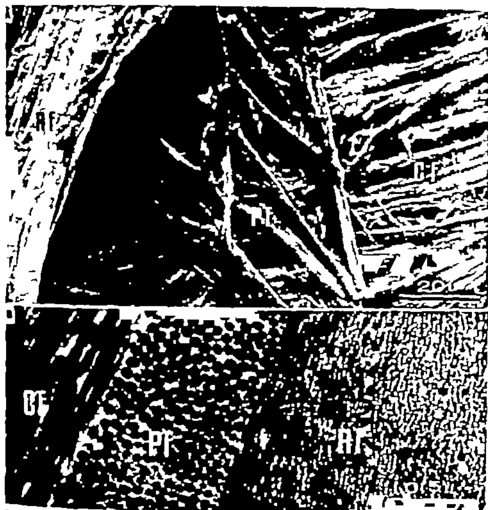


Fig. 6. (*A*) A scanning electron micrograph of the fiber layers in the pars tensa, showing that the parabolic fibers (*Pf*) are located between outer radial fibers (*Rf*) and inner circular fibers (*Cf*). The area photographed is the superior posterior quadrant. (*B*) Transmission electron micrograph of the fiber layers in pars tensa illustrates three different directional arrangements. Radial fibers (*Rf*) which are cross cut, parabolic fibers (*Pf*) and circular fiber (*Cf*) are longitudinally cut. The radial fibers are composed of finer fibrils intermixed with collagens.

and is much thinner than that of the pars flaccida. The epidermis can be subdivided into basal, Malpighian, granular and keratin layer. Occasionally a light cell which does not possess the characteristics of an epithelial cell can be found in the basal portion of the epidermis.

The lamina propria of pars tensa is composed of subepidermal connective tissue layer, outer radial fibers, inner circular fibers, a submucosal connective tissue layer and a mucosal layer (Figs. 4, 5). The subepidermal connective tissue layer and outer radial fibers are, in general, well-developed compared to the inner circular and submucosal connective tissue layer (Fig. 4). The latter layers in some areas of pars tensa are so poorly developed that they can no longer be traced. The subepidermal connective tissue layer is composed of loose connective tissue which is a continuation of the lamina propria of the pars flaccida. It contains abundant irregularly-arranged collagen fibers with a trace of elastic fibers. In this layer fibroblasts, capillaries, nerve fibers (myelinated and unmyelinated) are also found. Con-

trary to those in the pars flaccida, the nerve fibers in this part are mostly unmyelinated with an occasional bulb-like dilatation along its course Specialized sense endings were not observed in our material.

The morphology of radial and circular fibers is of great interest. Each fiber layer is formed of bundles of fibers which are mixed with varying amounts of typical collagen fibrils and finer fibrils" of unknown nature (Fig. 6)

The latter are much finer than the collagen and do not show typical banding of the col lagen in longitudinal section (Fig. 2 *B*) In cross section, this fibril is rectangular and suggests that it is formed of four sub-units (Fig. 2 *C*)

The mucous membrane of the pars tensa is composed of simple squamous cells. Occasionally lipid or fat droplets were found in the cytoplasm. The basement membrane is well developed in these cells, like those of the pars flaccida.

DISCUSSION

The ultrastructural characteristics of the human tympanic membrane are essentially the same as that of laboratory animals (Lim 1968 *a*, *b* Johnson et al., 1968) The pattern of fiber ar angement of the pars tensa and also the orphological characteristics of fibrils constituting these fibers are most interesting. Unlike that in animals, the fibrous layer of the human tympanic membrane is composed of a varying mixture of collagen and fine fibrils of an unknown nature The outer radial fibers contain more fine fibrils than the collagen, whereas, the inner circular fibers contained relatively more collagen. The typical collagen banding in longitudinal section was not observed in the fine fibrils and they were square in cross-sectional view Johnson et al. (1968) observed the same fibrils and their biochemical data further suggests that these fibrils are neither collagen nor elastin. Similar fibrils were observed in the connective tissue of the limbus spiralis, basilar membrane, and spiral ligament (Iurato & de Petris, 1960) of the vestibule (Hamilton, 1967) and in the human spiral ligament (Takahashi & Kimura, 1970) Chemical analysis and X ray diffraction of the spiral ligament (Iurato 1962) seemed to support the view that the fibrils are keratin. Although as was pointed out by Hamilton (1967), Iurato's finding did not include proline and hydroxy proline which would be diagnostic in clarifying between collagen and keratin. Ciges (1965), on the other hand, proposed that the fibrils are reticulin on the basis of histochemical staining characteristics However the validity of the silver staining" used in differentiating reticulin from collagen was challenged by the electron microscopic observation that the silver precipitated heavily even on collagen when their size was small (Banfield, 1958) Although the morphological characteristics as well as chemical characteristics of the reticular fiber is not clearly understood, it is generally believed that reticulin is closely related with collagen Further chemical analysis and X-ray diffraction should provide a clue on the nature of these fibrils

The existence of elastic fibers in human tympanic membrane was first described by Nishiyama (1937) and later confirmed by Wolff et al. (1957) However Hentzer's study (1969) failed to identify the elastic fibers in the lamina propria of human. Our material indicates that the lamina propria of pars flaccida contained an abundant amount of elastic fibers intermixed with irregularly-arranged collagen fibers, while, the pars tensa contained only a negligible amount of elastic fibers in the subepidermal layer near the pars flaccida. A similar-elastic fiber arrangement in the round window membrane was reported by Iurato & Recchia (1968)

Beside the radial and circular fibers in pars tensa the parabolic" and crescent fibers have been described (Filogamo, 1949 Kirikae, 1960 Fumagalli, 1942) Scanning electron microscopic (SEM) observation suggests that these parabolic fibers are located between radial and circular fibers. Their terminals fan

out and blend into the circular fibers. The details of the fiber arrangement will be reported in a separate paper (Shimada & Lim)

Shrapnell's membrane, contrary to the popular conception that it is only formed of two layers, distinctly possessed a well-developed lamina propria which is thicker than that of pars tensa, although lacking well-arranged fiber layers. The lamina propria of Shrapnell's membrane, as mentioned earlier is composed of abundant irregularly-arranged collagen and elastic fibers with numerous nerve fibers, both myelinated and unmyelinated, and blood vessels. The lamina propria also shows a loose arrangement with considerable ground substance which might account for the possible transparency of this membrane under clinical observation. These findings support the original description by Shrapnell (1832) that pars flaccida is more elastic than the pars tensa. Most current textbooks describe Shrapnell's membrane as thinner than the pars tensa. However Araki's (1944) and my findings point toward the fact that Shrapnell's membrane is actually thicker than pars tensa.

The epidermis of both pars flaccida and pars tensa was studied to see if there is any morphological difference which could account for the frequent development of cholesteatoma in case of marginal or Shrapnell's type perforation of the tympanic membrane It was suggested that papillary growth of the epidermis may contribute to a pearl formation of cholesteatoma (Schwartz, 1966) Although there was considerable individual variety the palisade appearance of the epidermal basal layer was readily noted in Shrapnell's membrane but less obvious in the tensa. Other than this occasional palisade appearance of the epidermis in pars flaccida, and the fact that the epidermis of pars flaccida is formed of many layers of desquamating epidermal cells, there was no other morphological evidence suggesting that they are different from the epidermis of pars tensa. A comparative study of the epidermis with that of the cholesteatoma will be reported separately (Lim et al to be published)

Although the mucosal lining of the tympanic membrane is basically formed by simple squamous cells with well-developed microvilli, occasionally the transitional area, where the tympanic membrane mucosa continues to the middle ear mucosa, is covered with stratified columnar or cuboidal cells with or without cilia. Intermixed with those ciliated cells, some cells resembling goblets were also noted. Similar observations were previously made by Lim (1968 *b*) among lower animals This finding may support the concept that the mucosal cell of the middle ear has the potential capability to be differentiated into secretory cells or ciliated cells

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ZUSAMMENFASSUNG

Submikroskopische Details des menschlichen Trommelfells wurden mittels eines Transmissionselektronenmikroskopes (T E. M.) und eines Scanningelektronenmikroskopes (S E. M.) studiert. Drei verschiedene Zellschichten wurden beobachtet: eine äussere epidermale Schicht, eine mittlere Lamina propria und eine innere Mucosa. In der Pars flaccida wird die mittlere Lamina propria aus lockerem Bindegewebe mit vielen lastischen und kollagenen Fasern gebildet. In der Pars tensa ist die mittlere Lamina propria hingegen aus äusseren strahlenförmig und inneren kreisförmig verlaufenden Fasern gebildet. Zwischen diesen zwei Faserzügen wurden uch parabolisch verlaufende Fasern beobachtet. Die Fibrillen der Pars tensa sind rechteckig im Querschnitt und sind mit verschiedenen vielen kollagenen Fasern gemischt. Im Gegensatz zu der verbreiteten Auffassung ist die Pars flaccida des Menschen, ähnlich wie in anderen Säugetieren, beträchtlich dicker als die Pars tensa.

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EXPERIMENTAL DEAFNESS CAUSED BY ETACHRYNIC ACID

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Abstract. A single large intravenous dose of etachrynic acid (20–40 mg/kg body weight) causes a rapid significant diminution in the voltages of cochlear microphonic potentials in the guinea pig. No permanent functional or morphologic alterations can be demonstrated 3 weeks following single large intravenous dose (30 mg/kg body weight) of the drug.

Etachrynic acid has been used as an effective diuretic agent in edema of cardiac, renal and hepatic origin for about 5 years. There have been several case reports describing acute transient hearing loss as a side effect of the therapy (Maher & Schreiner 1965 Schneider & Becker 1966 Pillay et al 1969) Even permanent hearing impairment resulting from this drug has been reported by Pillay et al. (1969) One recent report on human temporal findings seems to indicate that etachrynic acid therapy may cause loss of outer hair cells at the basal end of the cochlear duct (Matz et al., 1969) A feature common to all the reported cases was marked renal insufficiency Pure tone audiometrics have been recorded for several cases in which the hearing impairment was found to be more pronounced at higher audiometric frequencies Other audiological findings localizing the lesion in the cochlea or retrocochlearly have not been reported Preliminary results of animal experiments in the cat suggest that etachrynic acid may cause permanent hearing impairment in this species (Matz et al., 1969)

Some electrophysiological and histological findings in the guinea pig cochlea following administration of etachrynic acid will be reported here.

This study was supported by grants from the Finnish National Council for Medical Sciences and from the Paulo Foundation.

MATERIAL AND METHODS

The material consisted of 24 young guinea pigs weighing about 250 g, divided into four groups of 6 animals each. Etachrynic acid was administered as a single intravenous injection via a polyethylene catheter into the external jugular vein of the anesthetized animal. The solution contained 10 mg etachrynic acid in 1 ml physiological saline In three groups, the animals received a dose of 20, 30 and 40 mg/kg body weight, respectively To study the acute effects of the drug the animals in these three groups were examined and sacrificed 1 hour after the injection. The fourth group of animals received a single intravenous dose of 30 mg/kg body weight. These animals were examined 3 weeks later to study possible long-term effects of the drug.

The animals were anesthetized with Pentobarbital sodium (30 mg/kg body weight) injected intraperitoneally The bulla of the temporal bone was opened and a silver electrode (diameter 0.2 mm) was inserted in contact with the round window membrane (Lawrence et al 1959). The indifferent electrode was fixed to the subcutaneous tissue of the neck wound Pure tone signals from a generator (Bruel & Kjaer type 1024) at audiometric frequencies were delivered to the ears of t guinea pigs, using a hearing aid earpho

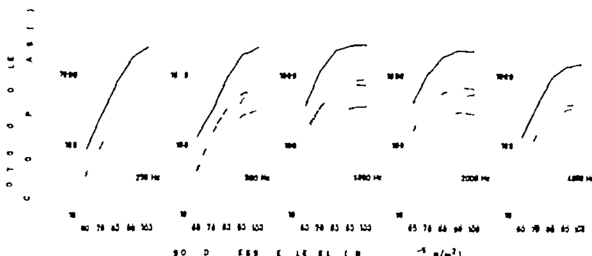


Fig 1 The mean voltages of cochlear microphonic potentials 1 hour after administration of etachrynic acid. —— recordings from the control animals, etachrynic acid 20 mg/kg body weight; 30 mg/kg body weight – 40 mg/kg body weight.

small plastic tube sutured to the external canal conducted the stimuli to the drum membrane The sound pressure levels at the end of the plastic tube were measured with a 2 cc coupler and a Bruel & Kjaer artificial ear (type 4152) The cochlear microphonic potentials were amplified by a Tektronix 122 preamplifier The voltages, peak to peak, were read by means of a frequency analyser (Bruel & Kjaer type 2107) in combination with a band pass filter set (Bruel & Kjaer type 1612) The control ' consisted of similar recordings from normal guinea pig ears.

After recording the cochlear microphonic potentials, the animals were killed and their cochleas fixed by perfusion with cold Veronal-buffered 1.5% osmium tetroxide solution. The cochleae were dissected and studied histologically under a phase contrast microscope according to the method of Engström et al (1966)

RESULTS

Acute effects

Fig. 1 presents the mean voltages of the cochlear microphonic potentials obtained for the three groups of animals which received a single intravenous dose of 20 30 and 40 mg/kg body weight, respectively and were studied 1 hour after administration of etachrynic acid. The *t*-test showed a statistically significant difference ($p < 0.05$) at each frequency and tone pressure level measured compared with the control recordings The mean voltages for the 20 mg/kg group of animals were generally slightly lower than those for the 30 mg/kg group however the *t*-test revealed no statistically significant differences between these two groups ($p > 0.05$)

Histologic study of the cochleae showed no pathological changes.

Chronic effects

The mean voltages recorded from 10 ears of the group of animals treated with a dose of 30 mg/kg body weight and studied at an interval of 3 weeks were statistically compared with the normal recordings. No statistically significant differences ($p > 0.05$) were found at any frequency or sound pressure level measured. No pathological changes appeared on histological examination of the cochleae.

DISCUSSION

A large single dose of etachrynic acid causes a rapid diminution in the voltages of cochlear microphonic potentials in the guinea pig, measured 1 hour after administration of the drug. The effect is more pronounced at the higher audiometric frequencies. In the long-term ex

periment there was no demonstrable diminution in cochlear microphonic potentials 3 weeks after a large single intravenous dose Histologic changes in the cochlea were not detected by light microscopy

Judged by cochlear microphonic potential measurements in the guinea pig, the hearing loss caused by etachrynic acid seems to be of cochlear origin It has been suggested that this drug, being a potent diuretic, may alter the electrolyte composition of the inner ear fluids and thus affect hearing. The presence of such a mechanism can only be proved by determination of the electrolyte compositions of the peri- and endolymph during exposure to the drug.

No long-term effects on the cochlea can be demonstrated in the guinea pig after a single toxic intravenous dose of etachrynic acid Further studies on other species of experimental animals are needed.

ZUSAMMENFASSUNG

Nach einmaliger Gabe von Etachrynsaure in hoher Dosierung (20-40 mg/kg Korpergewicht) kommt es beim Meerschweinchen zu einem signifikanten Spannungsabfall der Mikrophonpotentiale in der Cochlea. Bleibende funktionelle oder morphologische Veränderungen lassen sich dagegen drei Wochen nach einmaliger intravenöser Injektion von 30 mg/kg Korpergewicht nicht nachweisen.

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EVOKED RESPONSE AUDIOMETRY IN MENTALLY RETARDED CHILDREN

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Abstract. The auditory evoked responses of mentally retarded children were recorded using an averaging computer As a preliminary step, several anesthetics were examined for effectiveness with these children. The combined use of chloral hydrate and chlor promazine was shown to be most suitable while sodium methohexital was found to be most useful for those who were observed to be extremely hyper active. Although the minimal intensity for eliciting an evoked response was lower in older children, no difference in threshold was found among three dif ferent I Q classes. It is noteworthy that in children 5 to 10 years old there was a significant difference in peak latencies among three I.Q classes, but that after 10 years of age the difference was not significant. In comparing the below-fives with over fives (years of age) it ppeared that age differences also affected peak latencies. Some of the mongoloid hildren showed huge responses.

lthough the use of evoked response audiometry is now widespread and in most applications, satisfactory this technique is not without its problems. One of these is how to test uncooperative children who may be too immature, hyperactive, emotionally disturbed, epileptic or mentally retarded Accurate estimation of hearing ability can be helpful in making appropriate educational and social plans for these children. Such estimation can also be useful in identifying children whose hearing handicaps have caused them to be labelled incorrectly as retarded, aphasic, or emotionally disturbed. The primary purpose of the present study was to develop a method of testing the hearing of children who are unable to respond to auditory signals and with whom, for this reason, other methods are unsuccessful

There have been several reports in the past years concerning neurologic maturation or development retardation associated with response characteristics (Hogan & Graham 1967 Barnet & Lodge, 1967 Graziani et al 1968). However there are few references in the literature to research into the characteristics of evoked responses in retarded children. It was hoped, therefore that it would also be possible while developing a way of testing the hearing of such children, to investigate these responses in a systematic way

MATERIALS AND METHODS

Subjects

Two hundred children, aged 1 year 2 months to 17 years 1 month, all diagnosed as mentally retarded, were studied in two series of tests, one designed to produce data useful in the comparative study of some anesthetics which might be expected to be suitable for evoked response audiometry with hyperactive children, and another intended to permit observation of evoked responses in these children. Diagnoses of retardation were performed by physicians, psychologists, and social workers

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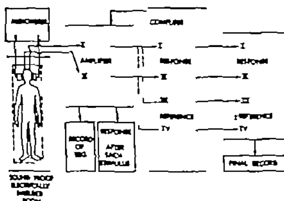


Fig 1 Block diagram of equipment.

in the Mental Retardation Centre, Toronto In cases in which physical findings were abnormal, the children underwent thorough preliminary check-ups by specialists.

Equipment

All tests were carried out in the EEG laboratory of the Mental Retardation Centre (Toronto)

Anesthetics studied were chloral hydrate, N_2O Penthrane, Fluothane, sodium methohexital, Ketalar (phencyclidine derivative CI 581), and chlorpromazine.

A block diagram of the equipment used in testing hearing is shown in Fig. 1 The sound stimulus used was a short tone burst of frequencies 500 1 000 2 000 or 4 000 Hz from a modified Madsen Electronics (Model OB60) Audiometer Its duration was 60 msec with a rise and decay time each of 20 msec. All subjects wore air conduction earphones the stimulus in which was calibrated to ISO 1964 standard using a Brüel and Kjaer Precision Sound Level Meter (Type 2203) with oscilloscope monitor In cases of suspected deafness, subjects underwent a further examination by means of a bone conduction vibrator in which the stimulus was calibrated to normal adult forehead threshold by repeated subjective testing. Sixty-four stimuli were presented at a rate of one every 2 sec, since preliminary experiments had indicated that this rate gave best results in lengthy testing under anesthesia.

Procedure

1 Comparative study of anesthetics

Chloral hydrate was used initially but was soon rejected because too much time had to be spent waiting for children to go to sleep N_2O Penthrane, and Fluothane were also tried, but with these no recognizable responses were obtained with averaging 32 and 64 times It was finally decided to compare chloral hydrate and two intravenous anesthetics (sodium methohexital and Ketalar) as against a combination of chloral hydrate and chlorpromazine (The best combination of the latter was an intramuscular injection of 1 mg per kg of chlorpromazine 30 min after oral administration of 30 mg per kg of chloral hydrate)

Under the direction of an anesthetist, the following experiments were performed.

Group A (10 children–20 ears) Tested with the chloral hydrate-chlorpromazine combination and retested with chloral hydrate

Group B (10 children–20 ears) Tested with the chloral hydrate-chlorpromazine combination and retested with Ketalar

Group C (20 children–40 ears) Tested with the chloral hydrate-chlorpromazine combination and retested with sodium methohexital.

2 Observation of evoked responses

At the beginning of each test sequence, two needle electrodes were placed symmetrically on top of each subject's head, 4 cm from Cz of EEG international electrode position on the line connecting both external auditory canals. A Beckman surface electrode was placed on the right mastoid as a reference electrode another Beckman electrode on the left mastoid was used as a grounded electrode. Electroencephalograms were continuously observed as a monitor of sleep stage by means of a Grass EEG Machine (Type III) Electroencephalographic averaging over a period of 1024 msec subsequent to the sound stimulus was done

Table I *Comparative study of anesthetics*

		Mean Test	Retest	Analysis of variance between test and retest
Group A	Threshold (dB)	23.4	20.9	$F = 0.35 < F$ 1/38 $= 4.10$ ($p = 0.05$)
10 subjects	Peak latency P_2 (msec)	252.9	251.5	$F = 0.03 < F$ 1/38 $= 4.10$ ($p = 0.05$)
20 ears	Peak latency N_2 (msec)	470.9	460.9	$F = 0.70 < F$ 1/38 $= 4.10$ ($p = 0.05$)
	Amplitude (μV)	61.1	61.7	$F = 0.003 < F$ 1/38 $= 4.10$ ($p = 0.05$)
Group B	Threshold (dB)	22.2	25.5	$F = 0.67 < F$ 1/38 $= 4.10$ ($p = 0.05$)
10 subjects	Peak latency P_2 (msec)	265.9	299.5	$F = 8.37 > F$ 1/38 $= 7.36$ ($p = 0.01$)
20 ears	Peak latency N_2 (msec)	464.1	481.8	$F = 1.58 < F$ 1/38 $= 4.10$ ($p = 0.05$)
	Amplitude (μV)	43.7	37.6	$F = 1.89 < F$ 1/38 $= 4.10$ ($p = 0.05$)
Group C	Threshold (dB)	23.2	20.0	$F = 3.54 < F$ 1/78 $= 3.97$ ($p = 0.05$)
20 subjects	Peak latency P_2 (msec)	274.6	276.9	$F = 0.008 < F$ 1/78 $= 3.97$ ($p = 0.05$)
40 ears	Peak latency N_2 (msec)	463.0	474.7	$F = 0.006 < F$ 1/78 $= 3.97$ ($p = 0.05$)
	Amplitude (μV)	40.7	49.2	$F = 3.42 < F$ 1/78 $= 3.97$ ($p = 0.05$)

a Fabritek 1052 Signal Averager Averaged evoked responses were transcribed onto graph paper by a Mosely 700A X Y recorder To facilitate detection of responses to weak stimuli, ± references from each channel and summated traces to no stimulus were used as controls. Threshold determinations were made in both ears at the four frequencies mentioned above Because the best response was obtainable in Stage 3 all responses were obtained in this stage, which is characterized by high voltage slow waves (delta waves) and some superimposed spindling.

RESULTS

Comparative Study of Anesthetics

Results of this investigation are shown in Table I. There was no difference between the effects of chloral hydrate with chlorpromazine and chloral hydrate alone in any point ($p > 0.05$) There was also no difference between the effects of sodium methohexital and those of the chloral hydrate–chlorpromazine combination ($p > 0.05$) Ketalar increased the peak latency of P_2 ($p < 0.01$) However it was quite difficult to get Stage 3 with Ketalar and sodium methohexital sent forth a good deal of spindling which sometimes interfered with responses. As a result, it was decided to test most of the children with the chloral hydrate–chlorpromazine combination, reserving the sodium methohexital treatment for extremely hyperactive cases.

Evoked Responses

In total 178 children were tested under anesthesia as described above, and 175 excellent sets of data were obtained. Three hundred and four ears of 153 children were considered to have normal hearing. These children were divided into three groups and three classes according to age and I.Q points (Table II) Of the 22 children who were diagnosed as having hearing impairment, two were found to have conductive hearing loss, 17 sensorineural hearing loss, and three moderately severe hearing loss (not further specified).

The responses of the mentally retarded children had five components (P_1 N_1 P_2, N_2 and P_3) of which the most stable were P_2 and

Table II *Classification of the subjects*
A = I.Q 79 to 50, B = I.Q 49 to 30, C = I.Q below 29

Age (years)	I.Q points A	B	C	Total
Below 5	15 (30 ears)	21 (42 ears)	13 (26 ears)	49 (98 ears)
5 to 10	22 (44 ears)	38 (75 ears)*	17 (34 ears)	77 (153 ears)
Over 10	13 (26 ears)	9 (17 ears)*	5 (10 ears)	27 (53 ears)

Two ears were not tested due t unsuitable sleep stage

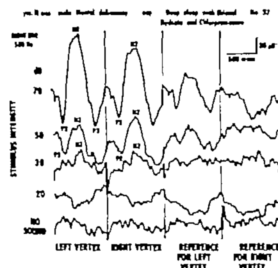


Fig 2 Evoked response audiometry under anesthesia.

N_2. In Stage 3 the amplitude of P_1 and N_1 decreased and the amplitude of N_2 and P increased markedly Peak latencies of P_2 and N_2 were stable, while peak latency of P_3 was quite variable Statistical analysis of the results was done, using an analysis of variance. All analyses were for the average of four frequencies data.

Thresholds

The threshold for evoked response audiometry was taken to be the minimal stimulus intensity which elicited a detectable cortical response It was easy to decide whether or not a small deflection was a real response by comparing it

Table III. *Threshold for auditory evoked response Mean and standard deviation of thresholds* (dB *Hl* ISO)

N = Number of ears tested

Age (years)	I.Q points A	B	C
Below 5	22.7±6.8 (*N*=30)	22.8±9.8 (*N*=42)	25.0±7.8 (*N*=25)
5 to 10	18.8±4.5 (*N*=41)	19.5±9 4 (*N*=73)	20 3±6.4 (*N*=34)
Over 10	12.0±2.8 (*N*=26)	12.3±4.0 (*N*=17)	16.9±8.6 (*N* 10)

Table IV *Peak latency of component P_2, me and standard deviation* (*MSEC*)

Age (years)	I Q points A	B	(
Below 5	282±24 (*N*=30)	265±34 (*N* 42)	67 5 (*N* 6
5 to 10	251±31 (*N*=44)	263±36 (*N*=75	275 18 (*N* 18
Over 10	254±18 (*N*=26)	258±28 (*N*=17)	6 1 (*N* 10

with a = reference which was obt d the same time as the two major r rd (2) The thresholds obtained are liste T III. While there was a significant differer of thresholds between age groups (p 0 0 no difference was observed between I Q ses ($p>0$ 05)

Peak latencies

The peak latencies of P_2 and N_2 of the evoked response were measured at 70 dB HL ISO (Tables IV V). There was no significant differences in peak latency between age groups or between I.Q classes ($p>0.05$) However there was a significant difference between I Q classes within the 5–10-year groups (P_2 $p<$ 0.05) (N $p<0$ 01) It is noteworthy that in the A Class of children there was a significant decrease over a 5-year period (between ages 0–5 and 5–10) in the latencies of both components P_2 and N ($p<0$ 01)

Table V *Peak latency of component N_2, mean and standard deviation* (*MSEC*)

Age (years)	I Q points A	B	C
Below 5	474±46 (*N*=30)	462±54 (*N*=42)	474±33 (*N*=26)
5 to 10	436±24 (*N*=44)	453±51 (*N*=75)	464±35 (*N*=34)
Over 10	435±41 (*N*=26)	446±51 (*N*=17)	466±22 (*N* 10)

Table VI *Amplitude (P_2N_2) of evoked response mean and standard deviation (μV)*

Age (years)	I Q points A	B	C
Below 5	43±11 (N=30)	45±17 (N=42)	51±20 (N=26)
5 to 10	42±11 (N=44)	50±23 (N=75)	48±17 (N=34)
Over 10	43±12 (N=26)	55±20 (N=17)	51±9 (N=10)

Amplitude

The peak-to-peak amplitude (P_2N_2) of the evoked response was measured at the intensity of 70 dB ISO (Table VI) There was no differences in amplitude between age groups or between I Q classes ($p>0\ 05$) It is noteworthy that eight of the eleven children with mongoloid features showed huge responses of more than 80 μV Three children with physical handicaps, two of whom were profoundly retarded, showed asymmetrical responses with amplitude differences of more than 30% on both hemispheres.

DISCUSSION

ce 1938 when Loomis et al. reported K mplex during sleep its characteristics have often been investigated in detail However K complex was only one of the indications of hearing in old fashioned EEG audiometry An application of the computer to EEG audiometry assigned a new part to the K complex—that of the most prominent component of the so-called "slow evoked response"

Because summated evoked slow response shows maximum amplitude and gives most reliable results during moderately deep sleep or deep sleep (Cody et al., 1967 Rapin & Graziani, 1967 Suzuki & Taguchi, 1968), we recorded every response during moderately deep sleep rather than light sleep To produce this state we used a combination of chloral hydrate and chlorpromazine, since chloral hydrate alone, while safe, is not strong enough to sedate a hyperactive child and chlorpromazine seems to strengthen and prolong its anesthetic effects. To maintain Stage 3 long enough to get satisfactory responses, it was necessary to add anesthetic little by little. In the course of this, two difficulties were encountered One was tachyphylaxis, which caused rapidly increasing resistance to the drug and necessitated administration of larger quantities of drugs as testing proceeded. This phenomenon depends markedly on individual differences in rates of metabolism which cannot be predicted in the absence of routine methods for rapid analysis of drug levels in a drop of blood (Conney 1969) Also of concern was an effect of therapeutic medication on the EEG Two subjects who were on Mellaril did not get to Stage 3 of sleep at all, either with the chloral hydrate-chlorpromazine combination or with sodium methohexital. In one case, sodium methohexital provoked convulsive EEG activity which interfered with evoked responses.

Because of its wide margin of safety and ease of administration Ketelar a new intravenous anesthetic introduced by McCarthy & Chen (1965) was investigated. However it was quite difficult to maintain an adequate moderately deep sleep for more than an hour with Ketalar alone. Also, Corssen & Domino (1966) have reported that visually evoked responses are depressed by this drug. There must be a relationship between evoked responses and background EEG pattern during sleep (Best & Tabor 1968) At this time, therefore it appears that the chloral hydrate-chlorpromazine combination method is best for hyperactive children, despite the fact that some difficulties are sometimes encountered in using it.

Rose & Rittmanic (1968) have reported using evoked response audiometry with some degree of accuracy with mildly to moderately retarded subjects with normal hearing as a clinical test for determining threshold, stating that in some individuals there was a significant discrepancy between voluntary audiometric and evoked response threshold. Davis (1965)

and McCandless & Lentz (1968) have reported obtaining evoked responses at near threshold during the waking state However there have been few references concerning threshold during sleep Although use of ± references (Schimmel, 1967) was helpful in detecting minute responses and ruling out possible false responses, threshold determination in our study was extremely time-consuming, requiring repeated tests. The results, however were interesting. The significant difference we found between age groups seems to suggest that some central development is taking place throughout childhood.

Peak latencies are supposed to be a sort of reaction time to stimuli. If any abnormality exists between the peripheral receptors and cerebral cortex, longer peak latency would be expected. Hogan & Graham (1967) have found that the evoked responses of mentally retarded adults were, on the average, longer in latency than those of a normal group Shimizu (1968) has presented results differentiating between cochlear and retrocochlear lesions by means of latency In our study we did not have normal controls. However it is interesting that in the 5–10-year-old group there were significant differences of peak latencies P and N among I.Q classes. There were still small, though not significant, differences among I.Q classes in the over 10 age group This seems to suggest that in the early childhood years greater development of the central nervous system takes place in the high than in the low I.Q group but that the low group may almost catch up with the high group after the age of ten. The irregularity of the below-five-years data is considered to be due to a great variability of peak latencies or to difficulty in determining I.Q levels accurately

Amplitude is the most variable factor in evoked responses. It depends heavily on sleep stage, age, maturation, and other factors. Barnet & Lodge (1967) reported that their mongoloid subjects had many more extremely large responses than their normal subjects. We observed the same feature in the responses of eight of our mongoloid su[illegible] related to the special patho[illegible] o[illegible] old brain—lack of differentiati[illegible] ous cortical areas, a decrease i[illegible] of cortical cells, and a reduction [illegible] of myelination (Book et al., 1959 [illegible] —pathological changes which woul[illegible] fective cortical control of centripetal impu[illegible]

It appears that the hearing handic[illegible] many children go undetected and th[illegible] result, many are wrongly believed t[illegible] tic, emotionally disturbed or retarded [illegible] once the hearing handicap and its degr[illegible] h[illegible] been established by means of a technique such as evoked respons[illegible] many children can be assisted w[illegible] aids. Even when a child is retarded, [illegible] of his hearing ability helps in plannin[illegible] future educational development and t[illegible] growth in areas of personal and social [illegible]petence

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ZUSAMMENFASSUNG

Die auditorisch hervorgerufenen Reaktionen an geistig behinderten Kindern wurden mit Hilfe eines ausgleichenden Computers untersucht. Zuerst wurden wirksame Betäubungsmittel für überaktive Kinder geprüft. Eine kombinierte Anwendung von Chloral Hydrat und Chlorpromazin wurde in solchen Fällen als geeignet empfunden, während sich für besonders überaktive Kinder Natrium Methobarital als wirkungsvoll erwies. Obgleich die minimalste Intensität, um eine auditorische Reaktion hervorzurufen, bei älteren Kindern geringer war konnte kein Unterschied im Schwellenwert bei den 3 verschiedenen Intelligenz-gruppen gefunden werden. Es ist bemerkenswert, dass bei geistig behinderten Kindern im Alter von 5 bis 10 Jahren bezeichnende Differenzen der Spitzenlatenz zwischen den 3 Intelligenz-gruppen feststellbar waren, dass aber bei über 10 Jährigen die Unterschiede unbedeutend wurden. Im Hinblick auf die Spitzenlatenzen ergab sich auch ein Unterschied zwischen Kindern unter und über 5 Jahren. Bei einigen mongoloiden Kindern wurden sehr grosse Reaktionen nachgewiesen.

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PRELIMINARY STUDIES OF GAS RESORPTION FROM THE MIDDLE EAR

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Abstract. ¹³³Xenon, dissolved in physiological NaCl, was introduced into the middle ear systems of 9 subjects with normal otoscopic findings. The activity over the middle ear system was measured with scintillation detector and the activity in the expired air was simultaneously recorded with separate, shielded detector In 3 tracheotomized cases the expired air was collected directly from the tracheal cannula. The recordings on living subjects as well as model experiment and measurements on dead subjects show primary phase of faster decrease mainly due to the mixing of xenon and air in the closed middle ear system. A secondary and slower phase represents the resorption and removal of xenon from the cavity This means that the decrease of activity of radioactive gas introduced into the ear does not reflect real absorption by the vessels in the mucosa before the end of a 8–10-minute period. The half life has been calculated. There was no correlation between the volume of the middle ear system and the disappearance rate of xenon. If the conditions of resorption are similar or identical for xenon and nitrogen, it seems possible to derive information about nitrogen resorption from data for xenon. From clinical as well as physiological point of view nitrogen is the most interesting gas in the middle ear system.

The middle ear and cellular system can be regarded as a closed, rigid chamber whose gas resorption presents special and complicated physiological problems of great clinical importance Respiratory physiologists have so far worked with elastic gas-pockets, especially in rats (Piper et al., 1962) but experiences based on such experiments are not quite applicable to the middle ear and cellular system. In the middle ear there is a slow continuous resorption of nitrogen and oxygen by the surrounding tissues. In a normally functioning system th volume of gas is replaced through the Eusta chian tube In various pathological conditions—e.g. acute and chronic catarrh of the tub —t. tubal function is impaired and an underp sure with subsequent oedema of the muc membranes and serous transudation develops As a consequence, hearing is impaired. It is therefore of interest to study the gas resorption —which we know comparatively little about—as a way to tackle clinical problems It is also interesting to find out if gas can diffuse through the tympanic membrane and if so, if the amount is of any importance compared with the amount passing through the Eustachian tube. Earlier investigations indicate that this is not the case (Riu et al., 1966 Elner 1970) In these preliminary studies we have used ¹³³Xenon which is chemically inert and fairly easily diffuses to the surrounding tissues.

METHOD

¹³³Xenon is a gamma radiating substance with a half-life of 5.3 days and its solubility in blood is about six times lower than that of nitrogen (0 8/100 ml blood) Xenon is practically cleared from the blood stream after one passage through the lungs. The gas was delivered in ampoules, dissolved in physiological NaCl-solution (The Radiochemical Center Amersham, England and AB

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Sweden). Xenon was introduced into the ear by puncturing the cellular system with a troicar through the skin under local anesthesia at the level of the spina supra meatum. This technique has previously been described (Flisberg et al., 1963; Ingelstedt & Rundcrantz, 1966). At the puncture the skin and subcutaneous tissue were displaced in relation to the underlying bone and periosteum. Before each puncture a roentgenological examination was made so as to exclude abnormal position of the sigmoid sinus or the dura mater. In all cases examined, tympanic membrane movements could be seen otoscopically when a slight over- and underpressure was produced with a small syringe. This showed that a free passage to the middle ear system through the needle existed. 0.10–0.15 ml of ^{133}Xenon, dissolved in physiological NaCl-solution, was injected and the needle was "washed" with a very small quantity of air. If any pressure in the ear was felt after the injection, the subject was told to swallow. The needle was in most cases taken out after the injection but measurements were also made with the needle lodged and sealed. The ear canal was sealed with a rubber disc in the bony part and with plastelina in the membranous part. In three experiments, performed on dead subjects, the ear canal was left open in one case, sealed in another and left open half the time and sealed half the time in a third. Measurements of the activity over the middle ear were made with a scintillation detector connected to a ratemeter. The detector was a crystal, 2 in × 2 in, with a cylindrical collimator (inner diameter 64 mm). The crystal was placed about 45 mm from the collimator orifice. The field "seen" by the detector covered the middle ear cavity and the cellular system. The measurements were started as soon as possible after the injection of xenon and always within the first minute. The activity was followed continuously with semilogarithmical or linear registration on a recorder of the potentiometer type. Time constant was 1–3 sec. The activity in the expired air was measured. The subject expired via an airtight rubber mask and a von Döbeln air-regulator into a rubber tube of fairly large diameter connected to a scintillation detector shielded by lead. The nose was clamped. The volumes of the cellular system have been calculated by planimetry of the X-ray picture (Flisberg & Zsigmond, 1965).

MODEL EXPERIMENT

The injection of xenon was made at a point in the cellular system which was not quite central. The scintillation detector was placed over the point of injection with its field of vision focused on the middle ear and the cellular system. In most cases the injection point was in the central field of the detector. This means that if the mixing of xenon and air in the cellular system is slow, the decrease in activity during the first minutes after injection reflects the process of mixing as well as the disappearance of xenon from the cavity, i.e. the resorption and removal of the gas. Later, when the concentration of xenon is more even, the decrease in activity will be a more accurate measure of the disappearance from the cavity.

To find out what happens when gases are mixed in a closed system of small communicating cells and cavities, a model experiment was made. A box of plexiglass (12 × 29 × 70 mm) was filled with glass beads of varying sizes (1–5 mm diameter). The volume of the air space in the box was approximately 16 ml. At the end there was a one way stopcock for the injection of xenon. On the inside, just in front of and approximately 5 mm from the stopcock, there was a small wall at a right angle covering 3/4 of the end area to screen off any jet effect of the injection. The same amount of xenon was injected as in the human subjects and two recordings were made, one with a fixed detector as in the human experiments, the other with a scintigraph. The scintigraph crystal passed over the box in 30 secs and during 40 min several scintigraphic profiles were recorded. The scintigraph orifice was a slit with a width of 2 mm.

Table I *Living subjects*

Subject	Diagnosis	Half-lif in min Per I	Per II	Volume of the middle ear system	Activity in the expired air	Comment
BW 34 06 03	Tinnitus	53	53	11 7	+	
AP 06 01 16	Tinnitus	55	138	16.6	+	
HN 06 05 29	Menière	50	80	9,5	0	
KH 09 08 29	Tinnitus	17	129	7 7	+	
SS 93 04 10	Normal	53	114	2.8	+	Tracheotomized
BP 43 03 23	Normal	80	189	20.4	(+)	Tracheotomized
FS 09 04 26	Tinnitus	84	233	7,0	(+)	
AS 10 11 08	Normal	27	31	6.1	+ +	Tracheotomized
TJ 15 04 26	Tinnitus	56	155	14 4	Not measured	
	Mean	53	124			

+ – activity over the background.
+ + – strong activity over the background.
(+) small activity over the background.
0 no activity over the background.

MATERIAL

The material consisted of 9 subjects, all of whom—except three tracheotomized patients—had aural trouble (For diagnoses see Table I.) The tympanic membranes were normal in all cases. The volume of the cellular system varied within rather wide limits (Table I) In three cases the measurements were made on tracheotomized subjects and the expired air was collected directly from the tracheotomy tube, with an inflated rubber cuff and von Döbein s air-regulator

Three recordings were made on dead subjects without history of ear disease and with normal otoscopic findings.

RESULTS

The model experiment shows that there is a continuous equilibration of the xenon concentration during the first 20 min This is shown in Figs. 1 and 2. There was at first a high intensity over that part of the model where the gas was injected but this decreased gradually as the activity in remoter parts of the model increased. The model was theoretically divided into three parts with the same volume. The distribution of activity between these parts was calculated by planimetry on the scintigraphical profiles (Fig. 2)

The figure shows the changes of activity in these three volumes in relation to time After 8 min there is a certain equilibration and after 20 min a fairly even distribution of activity in the model. In the experiments performed on dead subjects there was a decrease in activity during the first period of registration (8–10 min after the injection) In accordance with the model experiment this should correspond mainly to the spreading of gas in the closed cavity but after this period *no* decrease in activity could be measured (Table II) whether the ear canal was closed or not. In the experiment with a partly open and partly sealed ear canal there was no difference between the first and second part of the experiment. Table I shows the half-life for the different periods of

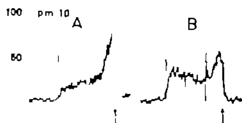


Fig 1 S. tigraphic profiles of the model (*A* mediatel te the injection of xenon, (*B*) after inje n The vertical lines theoretical d. ision of the model into volumes within different distance from injection This is marked by an arrow

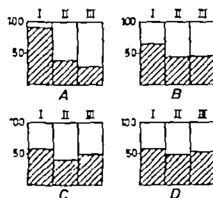


Fig. 2. The activity in the model at different time intervals after the injection. The three compartments were obtained by planimetry of the scintigraphic profiles shown in Fig. 1. (*A*) Immediately after the injection of xenon, (*B*) 8 min after the injection, (*C*) 20 min after the injection, and (*D*) 40 min after the injection. Compartment I nearest the site of injection.

nine persons and Fig. 3 a typical recording curve. In all cases the first period had the shortest half-life. Furthermore, Table I shows that in all cases except two a distinct activity was measured in the expired air.

DISCUSSION

The results of the measurements on the dead subjects show that it was possible to record only *one* primary phase of distribution. After that, there was no decrease in activity. When there is no circulation there is obviously no disappearance of xenon. A passive diffusion through the surrounding tissues is not likely to occur. The difference between the model experiment and the experiments on dead subjects—the half-life for the first period is shorter in the model—can be explained by a difference in temperature. The measurements on the dead subjects were performed at +4° to +10° C.

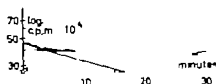


Fig. 3. Curve of activity from a living subject (KH Table I). Note the two tangents corresponding to periods I and II.

Table II. *Dead subjects*

No.	Half-life period I	Half life period II
1	130 min	Infinite
2	150 min	Infinite
3	130 min	Infinite

Period I = 0–8 min after injection of xenon.
Period II = > 8 min after injection of xenon.

the model experiment at normal room temperature.

In most cases the human experiments show a half life in the first period that is shorter than in the model and the dead subjects, but with fairly great individual differences. The most probable reason for this is that the kinetic energy of the gas increases with increasing temperature in the middle ear. *Fig. 3 shows that* after 8 min there is already a fairly good equilibration of activity in the model. With the more rapid mixing of xenon and air in the living subjects, the distribution can be regarded as fairly even after this time. The second period in the living subjects is therefore likely to represent a decrease in activity caused by the disappearance of xenon from the middle ear activity and the cellular system. Shortly after *the injection, activity in the expired* air was measurable and remained rather steady throughout the experiment. There is thus a continuous resorption of xenon during all the periods of measurement but the decrease of activity *in the ear* does not adequately reflect disappearance via the blood stream before the end of the first period, i.e. after 8 min. With the technique used in this investigation it is thus impossible to obtain information about *the absorption of xenon before* the end of an initial phase of mixing. It would have been desirable to make a quantitative determination of xenon in the expired air. However, this was impossible owing to variations in respiration and fairly low activity over the background. In the tracheotomized subjects the activity in the expired air was well in agreement with the rest of the subjects equipped with a rubber

mask and nose clamp. Activity in the expired air proves that xenon disappears from the ear via the blood stream. In some instances the subjects were told to swallow but in no case was there any change of activity in the ear or in the expired air. Leakage through the Eustachian tube is therefore negligible under normal conditions. This has also been shown by other investigators, (Riu et al., 1966). There was no observable leakage through the normal drum. It is therefore unlikely that the normal tympanic membrane contributes to the ventilation of the middle ear. The Eustachian tube plays the dominant role. There is no correlation between the size of the cellular system and the half-life of xenon. It is possible that gas resorption takes place mainly in the tympanic cavity which is lined with a columnar epithelium and to a less extent in the cellular system with its thinner and less vascularized mucoperiosteum (Flisberg, 1966). The absolute values of the volumes are perhaps too high. Using other methods other investigators (Ingelstedt, personal communication) have found lower values for the normal ear. The relationship between the different values, however, is not likely to be changed. The most interesting and important gas in the ear under normal physiological conditions is nitrogen, which makes up 80% of the total gas volume. If the area and path of diffusion is the same for nitrogen and xenon it should be possible—from the half-life values for xenon—to calculate the daily uptake of nitrogen in the normal middle ear since we know the diffusion coefficients for both nitrogen and xenon. The uptake of oxygen is more difficult to assess because of the metabolism in the mucous membranes and submucous tissues.

It would of course be of great interest to estimate the amount of blood perfusing the middle ear system, as has been done in the case of radioxenon in e.g. sceletal muscles. Unfortunately there is no method of determining the *area* of the mucous membranes in the middle ear system, which is essential for calculation of the blood flow. Knowing the approximate volume is not the key to this problem.

The exactness of the numerical values given in this investigation may of course be open to doubt, and certain sources of error might affect the interpretation of the curves. However the values given must be of correct magnitude. The decrease in activity has been given as the half life for a tangent drawn to the semilogarithmically plotted curve of activity without background subtraction. This might be criticized but has been found justified from a practical point of view.

ZUSAMMENFASSUNG

An neun Personen mit normalem otoskopischem Befund wurde ^{133}Xenon gelöst in physiologischer Kochsalzlösung ins Mittelohrsystem eingeführt. Die Aktivität über Ohr und Zellensystem wurde mit einem Scintillationsdetektor gemessen. Gleichzeitig wurde die Aktivität in der Ausatmungsluft mit einem gesonderten, geschützten Detektor gemessen. In drei Fällen mit Tracheostoma wurde die Ausatmungsluft direkt an der trachealen Kanüle gesammelt. Sowohl bei Versuchspersonen als auch in Modellexperimenten und bei Leichenversuchen konnte eine Anfangsfase mit schneller Aktivitätsabnahme festgestellt werden, die hauptsächlich darauf beruht, dass Xenon und Luft sich im geschlossenen Mittelohrsystem mischen. Eine zweite, langsamere Fase entspricht der Aufnahme und dem Abtransport von Xenon aus den Hohlräumen. Das bedeutet, dass das Nachlassen der Aktivität eines radioaktiven, ins Mittelohr eingeführten Gases erst nach Ablauf von 8–10 Minuten einer wirklichen Aufnahme durch die Schleimhautgefässe entspricht. Die Halbierungszeit ist berechnet worden. Es konnte kein Zusammenhang zwischen dem Rauminhalt des Zellensystems und der Abnahme von Xenongas festgestellt werden. Vorausgesetzt, dass die Bedingungen für eine Aufnahme von Xenon und Stickstoff ähnlich oder identisch sind, besteht die Möglichkeit einer Schlussfolgerung hinsichtlich der Aufnahme von Stickstoff aus den durch Xenonmessungen erhaltenen Resultaten. Stickstoff ist sowohl vom klinischen als auch vom physiologischen Standpunkt wahrscheinlich das interessanteste Gas im Mittelohrsystem.

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THE MECHANICS OF LARYNGEAL FUNCTION

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Abstract. Mechanics of the internal movement of the larynx are based mainly on laryngeal articulations, muscles and their nervous control. A review of the present concept of mechanics of the crico-arytenoid and the crico-thyroid joints suggests a re-evaluation of the action of various laryngeal muscles, internal and external. Percutaneous electromyographic study of various laryngeal muscles invites modification of the concept of the action of the different functional groups of internal laryngeal muscles. The influence of the external laryngeal muscles on the size and configurations of the laryngeal chink is also stressed. A review is given of the different considerations of the mechanism of action of the external laryngeal muscles on the vocal folds position and tension. A short survey of the motor control and afferent outflow of the larynx is presented. This demonstrates the complexity of the nervous control of the larynx and the uncertainties concerning the details of its function.

The larynx is functionally constructed from sets of skeletal muscles playing on certain joints and supplied by some cerebro-spinal nerves. The final clinical outcome of the resultant of all these factors decides the position and configurations of the vocal folds as seen at laryngoscopic examinations.

Considering the current discussion on laryngeal articulations it has been found that the crico-arytenoid joint is one of the most controversial and least understood joints in the entire body The conventional textbook concept of arytenoid rotation is illustrated by comparing the cross section of each arytenoid to a right angled lever Each arm of the lever represents one of the processes of the arytenoid. Displacement of one arm of the lever follows displacement of the other and this movement occurs around the fixed center of the lever through which passes the assumed vertical axis of the arytenoid mass

It should be admitted that such a theory ignores the basic anatomical relations of the articular surfaces of this joint. As described by Frable (1961) the average dimensions of the articular facets are (*a*) 3.2 × 4 mm across the concave arytenoid facet; the long axis of the facet is in a parasagittal plane of the body (*b*) 2.8 × 6 mm across the cylindrical cricoid facet the long axis of this facet passes through the cricoid parallel to its upper border This axis meets the median sagittal plane of the body at an angle of about 45 degrees, and the horizontal plane through the lower cricoid margin at almost the same angle. The cricoid surface is appreciably longer and somewhat narrower than that of the arytenoid. At no position of the joint do the two facets accurately coincide If the conventional concept of rotation about the vertical axis of the arytenoid mass were possible there would be no need of an articulation of the shape which exists.

This rotational theory though universally accepted, has been the subject of many refuting publications. As early as 1866 Henle denied the theory of arytenoid rotation about a vertical axis. Snell (1947) did not accept the existence of a vertical axis and felt that motion around this axis was checked in either direction by the posterior crico-arytenoid liga-

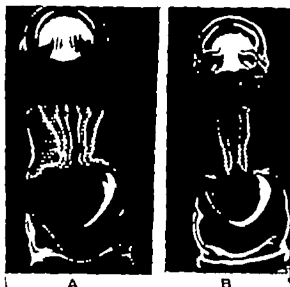


Fig 1 The two main types of movement occurring at the crico-arytenoid joint are (*A*) Rocking around an *oblique* axis, and (*B*) a linear glide parallel to this axis. (Modified from von Leden & Moore, 1961).

ment. He assumed that the axis around which the arytenoid cartilage moves coincides with the longitudinal axis of the cricoid facet. The notion that laryngeal closure is associated with a downward and forward turning movement of the vocal folds together with the vocal processes has been expressed by Curry (1940) This observation was considered by Ardran et al. (1953) to be incompatible with rotation of arytenoid around a vertical axis.

von Leden & Moore (1961) suggest that the structural arrangement of the crico-arytenoid joint permits two principal types of motion. (*a*) A rocking or rotatory movement around the axis, and (*b*) a linear glide parallel to this axis (Fig 1)

The former movement provides the main leverage for the massive movement of the vocal folds during opening and closure of the glottis. Th axis of rotation is an oblique one from *dorso-medio-cranial to ventro-latero-caudal* positions The plane of rotation of the cartilage is primarily dorso-latero-cranial to ventro-medio-caudal. The linear glide is more limited in extent. *It follows the longitudinal dimensions of* the cricoid facet. In isolation this movement tends to shorten or lengthen the vocal fold during vocal adjustments with a small amount of lateral displacement.

Ardran & Kemp (1966) utilizing still radiography ciné fluorography and *tomography* found that during passive displacement of the arytenoids, when the glottis was opened, the antero-posterior projection showed the arytenoids situated upon the top of the cricoid facets inclined about 40 degrees outwards. When the glottis was closed the arytenoids were situated upon the lower and medial aspects of the facets aligned with their axes approximately parallel to the middle line These authors found that glottic closure is dependent upon apposition of the vocal processes consequent upon the rocking movement of the arytenoids downwards, *forwards* and inwards. There was no approximation of the bases of the two cartilages upon the back of the cricoid, as was generally believed Studies on active movements in the living gave almost identical results

Considering this concept of the mechanics of the crico-arytenoid opening and closure it is believed that the already disputed role of the posterior crico-arytenoid muscle as the main glottic opener becomes even more doubtful The demonstration of an upward movement of the arytenoid over the cricoid in glottic opening puts the posterior crico-arytenoid muscle in an unfavourable mechanical situation This muscle can produce backward and a slight outward movement, but it is quite unlikely that the muscle could effect an upward displacement of the arytenoid over the cricoid. Such a displacement is probably due to a force applied to the arytenoid mass from above

Furthermore it is clear that movement of the cricoid cartilage relative to the thyroid cartilage at the crico-thyroid joint will influence the effects of arytenoid movement, since movement of the cricoid displaces the arytenoids bodily The movement that takes place at the crico-thyroid joint is mainly rotation around a transverse axis that passes the centres of the two joints. Thus the antero-superior border of the cricoid and the antero-inferior thyroid border

can be approximated to a visor-like movement at the crico-thyroid joint.

Radiological studies (Ardran & Kemp 1966) point out that the visor is partly opened at rest. It closes on phonation, especially notes of high pitch. During swallowing, straining or any act in which the larynx closes, this visor is usually widely opened, with shortening of the vocal folds. If we assume that closure of the visor is achieved only by the crico-thyroid muscle we are likely to face a functional paradox. The crico-thyroid, known to be a vocal fold adductor is thus contracting during swallowing, while the visor is widening. Consideration on a wider scale of the system of forces acting on the crico-thyroid joints will bring about a satisfactory explanation.

Zenker (1964) stated that all forces operating in front of the axis of the crico-thyroid joint, on the cricoid in an upward direction, and all forces operating behind this axis downwards, lead to closure of the visor and elongation of the vocal folds. On the other hand, all forces active in front of this axis, which pull the cricoid cartilage downwards, and those effective behind the axis which pull in an upward direction, will open the visor and shorten the vocal folds (Fig. 2) It has been suggested by Schilling (1937) Sonninen (1954) and Ardran & Kemp (1966) that the sterno-thyroid muscle is able to effect widening of the visor by tilting the thyroid cartilage backwards. In an analogous way the thyro-hyoid muscle is capable of widening the visor According to the same principle the lifting up of the larynx by the longitudinal pharyngeal muscles, in the second stage of deglutition, leads to an increase in tracheal traction, which opens the visor It is thus evident that some distant muscles such as the sterno-thyroid and some elements of the *functional chain* can influence the position and configuration of the vocal folds. Accordingly any consideration of laryngeal muscular function should be considered incomplete without a thorough study of the role of the external laryngeal muscles.

Contrary to the external laryngeal muscles

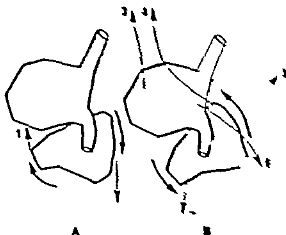


Fig Forces acting on the ... Closure of the ... (A) is achieved ... thyroid muscle and ... Opening of the visor (B) is brought about by ... *4 functional chain* of arytenoid, ... fold, epiglottis, hyoid bone and ... pharyngeous muscle 6, sterno-thyroid ... 7 tracheal tension. (Modified from ...)

the small internal muscles of the larynx have been the subject of many intensive studies. It is generally accepted that the only abductor of the glottis is the posterior crico-arytenoid muscle while the rest of the internal laryngeal muscles are considered to be a group of adductors This concept of function is based on mechanical analysis of the muscles pull on the joints Many of the assumed facts concerning the mechanics of these joints have already been declared fallacious It has been concluded that the concept reflecting optimally the physiological state will be obtained from studying the action potentials of these muscles in man, while performing different actions

In an attempt to fulfil this task percutaneous electromyographic recording was obtained from various laryngeal muscles representing the different functional groups (Faaborg ...). The action potentials recorded in this way were generally of low amplitude (50 ... µV) and short duration (–4 msec) (Fig. 3) These features can be explained on the ... innervation ratio

A slight degree of resting electrical ... was found in the examined mus...

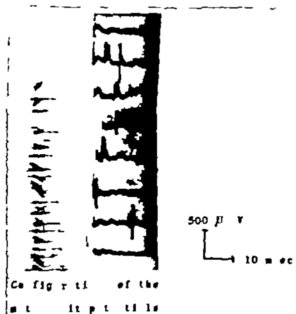


Fig 3 The parameters of the laryngeal motor unit action potentials are of short duration (2–4 msec) and low amplitude (50–850 μV).

the larynx. This denotes that these muscles are in a state of constant tonic contraction This activity can be considered as postural in nature and may be provoked through a proprioceptive mechanism (Faaborg-Andersen, 1957) The constant pull of these muscles will have a stabilizing effect on the crico-arytenoid joints.

During inspiration this resting electrical activity increased in frequency and amplitude while it was not affected by expiration (Kotby 1967) During forced inspiration the increase was still greater It was exhibited to almost the same extent by all muscles tested, whether abductor or adductor This is considered paradoxical with the so-called adductor muscles. Yet, it can be accepted as an effort on their part to stabilize the crico-arytenoid joint and to stiffen the edges of the vocal folds, thus preventing their flapping and collapse in front of the incoming jet of air during inspiration.

On performing sphincteric functions it could be seen that the adductors reached their maximal electrical activity The activity recorded from the posterior crico-arytenoid muscle in glottic sphincteric actions may be explained as a balancing force which counteracts excessive forward pull produced by contraction of the thyro-arytenoid and crico-thyroid muscles on the arytenoid

It was noted that during emission of sound all laryngeal muscles show a considerable degree of electrical activity maximal in the thyro-arytenoid and the crico-thyroid, which are the tensors of the vocal folds. The rest of the adductors help to close the larynx, to build up subglottic pressure an essential requirement to set the vocal folds into vibrations to emit sound. No convincing explanation can be given to the role played by the posterior crico-arytenoid muscle

The results reached upon investigation of the posterior crico-arytenoid muscle electromyographically by a percutaneous technique were so contradictory to the current concept that a confirmatory EMG study of this muscle under direct vision was considered necessary Some preliminary results of this study have confirmed the inspiratory rise of electrical activity in the posterior crico-arytenoid muscle (Fig. 4) In addition, the muscle showed a similar and almost equal rise of electrical activity during phonation and straining (Fig. 5) The latter two actions entail forcible adduction of the vocal folds, with an expected return to the resting electrical activity of the sole abductor

The sterno-thyroid muscle, a member of the extrinsic laryngeal group, has been demonstrated to exhibit a pronounced electrical activity synchronous with inspiration (Fink et al., 1956 Kotby 1967) It was assumed that this inspiratory activity denotes that the muscle has an influence on widening the glottis, thus introducing a possible influence of the extrinsic laryngeal muscles on glottic size and configurations.

In general the external muscles serve to fix the larynx and bring about its vertical movement. More specifically the external muscles can influence the shape and size of the glottis in different ways. Zenker (1964) noted that the arrangement of the mucous membrane and connective tissue of the larynx allows any ver

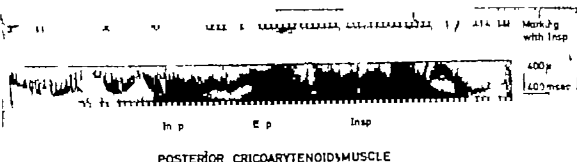


Fig 4 The posterior crico-arytenoid muscle shows rhythmic increase of electrical activity with inspiration. Marking indicates onset of inspiration as judged by the thoraco-abdominal movement. *N.B* The marking is delayed as compared with maximal inspiratory activity due to delay in the observer's appreciation of mass movement and his response by pressing the button.

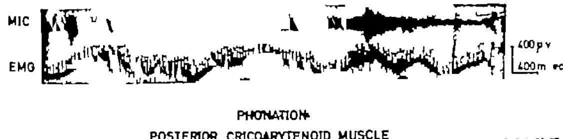


Fig 5 Synchronous increase of electrical activity in the posterior crico-arytenoid muscle (*lower trace*) with phonation of "E" as recorded by microphone (*upper trace*) placed in front of the mouth.

tically applied force to be effective as a glottis opener In this way the tracheal pull during inspiration, especially if the base of the tongue is fixed or even more is raised, brings about widening of the glottis to a noticeable effect. Fink et al. (1956) suggested that laryngeal depressors, specifically the sterno-thyroid, are imparting a stretching force in a downward direction on the ary-epiglottic fold. In other words, the lower end of the *functional chain* of arytenoid, ary-epiglottic fold, epiglottis, hyoid bone and tongue, is pulled down while the upper end is fixed. The ary-epiglottic stretch will produce an upward and outward pull on the arytenoid cartilages, which are drawn laterally carrying the vestibular and vocal folds away from the middle line (Fig. 6) This effect of pull on the *functional chain* is in accordance with the observations of Ardran & Kemp (1966) on the mechanics of the crico-arytenoid joint. In addition, Fink et al. (1956) assumed that the descent of the larynx causes a stretching of the pre-epiglottic body in the anterior wall, thus increasing the antero-posterior diameter during inspiration. Moreover during descent of the larynx the folds on its lateral walls become stretched and unfolded in a way similar to the walls of a bellow In this way on descent of the larynx its lumen is enlarging in all directions Correspondingly Zenker (1964) noticed that the effect of laryngeal elevators reverses the process They narrow the laryngeal vestibule, not only by pushing forward the epiglottis, but also by bulging the lateral vestibular walls inwards and backwards towards the arytenoids.

For a long time much attention was given to the influence of the strap muscles on pitch control and vocal fold tension. It should be stressed that tension of the vocal fold is not a tight stretching of the cord like the tuning of a violin string, but rather a firming of the

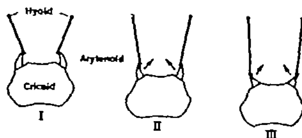


Fig 6 The descent of the larynx (cricoid cartilage with the arytenoids on top) leads to stretching of the ary-epiglottic folds. The latter have one fixed point to the hyoid bone through the hyo-epiglottic ligament. This stretching will exhibit a pulling force on the arytenoid cartilages, moving them upwards and outwards over the cricoid facet. (Modified from Fink et al., 1956.)

vocal fold's edge produced by the muscular support of the fold. In other words, tension of the vocal fold is not only a function of its length. In muscular folds, as in man, length and tension are determined by separate mechanisms. Length is determined by the crico-thyroid muscle and probably by all forces acting on the crico-thyroid visor in either direction. Tension is determined by the intensity of contraction of the thyro-arytenoid muscle, which is dependent on its muscle fibre length, mber of motor units activated and frequency motor impulses (Hast, 1966) The active etric tension exhibited by a striated muscle is greatest at the resting length of the muscle fibre, defined as the maximal length of the fibre in the body In case of the thyro-arytenoid muscle the respiratory length was found to be only 65% of the maximum phonatory length, which represents the physiological resting length at which isometric tension is developed most efficiently (Fink, 1962)

This illustrates the importance of the forces acting on the vocal fold length, which are considered to provide the crude vocal fold tension of isotonic type that enables the thyro-arytenoid to exert fine tension of isometric type. The involvement of the external laryngeal muscles in pitch control is thus their effect on the crico-thyroid visor hence on vocal fold length. Schilling (1940) has emphasized this role in the *frame function theory* which has been later modified by Sonninen (1956) This theory assumes that an antagonism exists between the crico-thyroid and sterno-thyroid muscles Suspended between these two muscle systems the thyro-arytenoid would be in a state of balance to carry out its function of shaping the glottis for various phonic performances. The crico-pharyngeous muscle which is a vocal fold shortener may produce, when acting against the crico-thyroid muscle, a similar effect on the thyro-arytenoid muscle. In extremely low pitches, when the vocal fold has its shortest phonatory configuration, Zenker (1964) has noticed that the crico-pharyngeous, and to a lesser extent the crico-thyroid, are called into play

Confirming these assumptions is the observation of vocal disorders following lesions and injuries of the laryngeal depressor-elevator mechanism. A peculiar type of transitory vocal disability following thyroidectomy has been described by several authors (Sokolowsky 1936 Schilling, 1937 Luchsinger 1942) This has become known as dysphonia following strumectomy without laryngeal paralysis." It was attributed to either severence of the strap muscles on one or both sides or to paralysis of these muscles. Arnold (1947) described a case of dysphonia following bilateral severence of the hypoglossal nerves. In this connection it is stressed that paralysis of a vocal fold does not always necessarily entail loss of its massive movement. It may as well mean the loss of function of some muscles which control its fine internal movements.

Vocal fold tension thus adjusted peripherally by the various sets of muscles is constantly checked through feed-back circuits conveying data to the central control which tunes the degree of muscle contraction. In this way what is known as the peripheral myo-elastic theory of voice production, in contradistinction to Husson's (1950) central theory is not only peripheral, since the central nervous system imparts a great deal of tuning effect. The feed-back circuits include auditory pathways and

proprioceptive channels via afferent laryngeal nerves.

The efferent innervation of the larynx starts in the cerebral cortex at the foot of the frontal precentral gyrus. The axons of the primary neurons run to the medullary centres via the corona radiata and travel in the internal capsule at the junction of the genu and the posterior limb The fibres then descend mainly in the cortico-bulbar component of the pyramidal system. These fibres project on the bulbar nuclei of both sides

The cell bodies of the secondary neurons lie in the nucleus ambiguus in the caudal part of the brain stem. The cranial third of the nucleus ambiguus is considered to be the somato-motor center of the pharyngeal and some of the palatal muscles. The center for the crico-thyroid muscle is closely related to the previous one This illustrates that the crico-thyroid muscle is functionally incorporated with members of the external laryngeal muscles The peripheral fibres supplying the crico-thyroid muscle are distributed via the external branch of the superior laryngeal nerve. The caudal parts of the nucleus ambiguus regulate the intrinsic laryngeal muscles attached to the arytenoid cartilage. All these muscles have been shown to act in intimate harmony The peripheral fibres supplying these muscles are distributed in the inferior or recurrent laryngeal nerve In spite of this segmental motor innervation of the palate, pharynx and larynx, it is considered that the fibres supplying the larynx originate in the bulbar portion of the accessory nerve The recurrent nerves, which are branches of the vagus nerve, thus contain fibres of accessory origin.

There have been several attempts to describe a fixed pattern of arrangement of nerve fibres in the recurrent nerve trunk. The fibres supplying the posterior crico-arytenoid muscle have been thought to occupy a peripheral position. The work of Sunderland & Swaney (1952) on the intraneural topography of the recurrent nerve failed to confirm any constant pattern. They found that the nerve bundles were not arranged in parallel strands, but that there was an intermingling of fibres from different fasciculi.

The recurrent nerves enter the larynx immediately behind the inferior cornu of the thyroid cartilage They may enter the larynx unbranched or they may divide into two to six branches at a variable distance from the crico-thyroid joint (Bowden, 1955) Separate nerve bundles destined to certain muscle groups have not been traced

The fibres of the recurrent nerve end on a small number of muscle fibres, from ~3 (Rüedi, 1959) up to 30 (English & Blevins 1969) Such a low innervation ratio is striking when compared to other skeletal muscles, which may have up to 2 000 fibres per unit. This neuro-muscular arrangement of the laryngeal muscles is suitable for the action required from these muscles. They are known to be non weight bearers and to perform delicate actions entailing rapid contractions.

On approaching the muscle fibres the motor nerve fibre loses its myelin and Schwann sheaths and comes into close contact with the sarcoplasm. At this level the cytoplasmic membrane creases into a particular structure called the subneural apparatus (Piquet & Barets, 1960) Laryngeal muscle fibres show more than one end plate, large and small, located at various unfixed sites, in many cases at one end of the fibre The nerve fibres supplying the small end plates, without a characteristic subneural apparatus, have been found to be of a thin calibre (Zenker 1964) an arrangement suggesting a similarity to the intrafusal muscle fibres of the gamma-motoric system

The sensory outflow from the larynx above the level of the glottis is served by superior laryngeal nerve fibres The recurrent laryngeal nerve carries sensory stimuli from the lower part of the organ A number of sensory receptors have been described in the laryngeal structures of mammalian species. These are mucosal touch receptors and mechanoreceptors in the joint capsules, as well as in the deeper structures of the larynx (Eyzaguirre et al., 1966) The mechanoreceptors in the larynx are

of two types, articular mechanoreceptors of phasic excitation and the receptors responsible for the myotatic reflexes (Abo-El-Enein & Wyke 1966) Muscle spindles have been long denied to exist in the intrinsic laryngeal muscles It was even thought that this group of muscles, being non-weight bearers, are simple in action and hence have very little proprioceptive supply This is quite contradictory to the constant finding of resting postural activity in these muscles. Some workers (Lucas Keene, 1961 Bianconi & Molinari, 1962) have repeatedly demonstrated, however the presence of muscle spindles in intrinsic laryngeal muscles of man and animals.

The proprioceptive outflow of the larynx is strictly unilateral and is served mainly by the superior laryngeal nerve (Eyzaguirre et al., 1966) The recurrent nerve alone is, nevertheless, capable of conducting efferent and afferent stimuli, since there is evidence that the diameter spectrum of motor fibres to laryngeal muscles is very wide (Murtagh & Campbell, 1951) Therefore, one would be tempted to assume that two independent motor systems may be present, one innervating muscle receptors and the other supplying ordinary muscle fibres The cell bodies for the primary ron in the afferent system are probably ated in the jugular ganglion of the vagus erve

From the preceding discussion it is evident that the neuro-muscular system influencing vocal movement is far from being simple and by no means clearly understood. It seems, as Zenker (1964) stated, that we have before us a key-board upon which we are capable of playing in an enormous variety of different ways.

ZUSAMMENFASSUNG

Die Mechanik der inneren Bewegung des Kehlkopfes gründet sich hauptsächlich auf die laryngealen Gelenke, Muskeln und ihre nervöse Kontrolle Ein Überblick über die jetzige Auffassung der Mechanik des cricoarytenoiden und des crico-thyreoiden Gelenks fordert zu einer Umwertung der Wirkung der verschiedenen inneren und äusseren Kehlkopfmuskeln uf. Perkutanes elektromyographisches Studium von verschiedenen laryngealen Muskeln ladet zu einer Modifikation der Auffassung über die Wirkung der verschiedenen funktionellen Gruppen der inneren Kehlkopfmuskeln ein. Der Einfluss der äusseren Larynxmuskeln auf die Grösse und Form der Stimmspalte wird auch betont. Es wird ein Überblick der verschiedenen Auffassungen über den Funktionsmechanismus der äusseren Larynxmuskeln auf die Position und Spannung der Stimmenfalten gegeben. Eine kurze Darstellung der motorischen Kontrolle und der afferenten nervösen Regulierung des Kehlkopfes wird gegeben. Diese zeigt den komplizierten nervösen Kontrollapparat des Laryn und die Unsicherheiten betreffend der Detaillen seiner Funktion.

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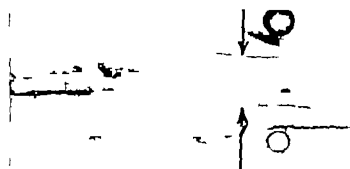
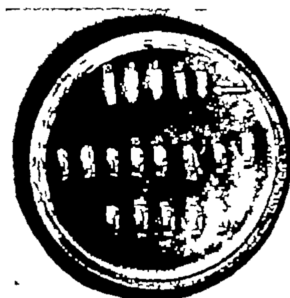


Fig 1 An immunophorogram of the nasal mucous membrane secretion in allergic rhinitis, (*a*) (*upper*) homogenized secretion, (*b*) arrow indicates the third, non-recognized fraction.



}2 Designation of fibrinolysis areas on fibrin *ates* in relation to distribution of agar strips containing the electrophoretically separated secretion of the nasal mucous membrane.

A further step in our experiment was an attempt to identify the protein fraction that showed fibrinolysis and occurred within alfa$_2$ beta$_1$ group

METHODS

The specimens were collected, as already described, and examined for fibrinolytic activity: they were then studied by immunophoresis using monovalent sera. anti-beta-lipoprotein, anti-alfa$_2$ macroglobulin, anti-transferrin, anti-Gc globulin, anti-beta$_{1AC}$ globulin, anti-haptoglobulin and anti-coeruloplasmin (Behring Werke, West Germany)

RESULTS

The sera used failed to produce precipitation with the protein constituting the "third fraction"

As already mentioned, the experiments were performed in two large groups, i.e. with homogenized and non-homogenized samples *Homogenization was found to have no effect on fi*brinolysis, whereas it did cause a reduction in the sharpness of albumin arch with concomitant increase in the prominence of the "third non recognized" fraction in the immunophorogram.

Summing up the above given findings, we could establish in the secretion of the nasal mucous membrane, in allergic rhinitis and in health the presence of a protein factor displaying an electrophoretic mobility of alfa$_2$ and beta$_1$ globulins and a certain proteolytic activity

The difficulties we met in attempts to identify the "third protein fraction" though it has been detected with polyvalent, serum protein precipitating serum, warns against any binding conclusion.

Our further studies will be concerned with.

1 detailed characteristics of the fraction referred to as "third fraction

2 assessment as to whether fraction is merely the carrier of a protelytic enzyme or constitutes the whole of it.

ZUSAMMENFASSUNG

Die Nasenschleimhautsekretion wurde bei 10 Gesunden und 49 Kranken mit allergischer Rhinitis untersucht. Es konnte nachgewiesen werden, dass die Nasensekretion von beiden Gruppen proteolytische Eigenschaften besitzt, diese sind beider letzteren Gruppe mit der Fraktion verbunden, deren elektrophoretische Mobilität der von Alfa$_2$, Beta$_1$-Globulinen entspricht, und die im Elektrophorogramm einen charakteristischen Präzipitationsbogen zeigt. Das Eiweiss ist bezeichnet als „dritte" Fraktion.

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ILT[illegible] [illegible]CTURAL FINDINGS IN A CASE OF BENIGN [illegible] THELIAL LESION (SJÖGREN'S SYNDROME)

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(Received May [illegible]2, 1970)

[illegible] because [illegible] region [illegible] formed [illegible] imen [illegible] clin- [illegible] [illegible] ultra- [illegible] con- [illegible] ry [illegible] replaced [illegible] by pro- [illegible] one of [illegible] by cytoplasm [illegible] terized [illegible] organelles and [illegible] numerous [illegible] Other island cells [illegible] filaments, [illegible] plasm tonofil[illegible] [illegible] dense cyto- [illegible] addition, there [illegible] somes. In [illegible] features intermediate [illegible] that possessed [illegible] the two other kinds of cells. N[illegible] [illegible] m[illegible] epithelial cells were found. Although conclusive [illegible] evidence is lacking, it is believed that the cell islands might be formed by proliferation and squamous cell metaplasia of duct cells.

The light microscopic alterations in the salivary glands are identical at Sjögren's syndrome, Mikulicz's disease and benign lymphoepithelial lesion (Godwin 1952, Morgan 1954, Seifert & Geller 1957, Grage & Lober 1964, Bark & Perzik, 1968). Proliferations of the ducts with the appearance of myoepithelial cell islands have been suggested to be pathognomonic for these conditions that often are referred to as myoepithelial sialadenitis (Seifert, 1966, Ericson, 1968, Ericson & Sundmark, 1970). Recent investigations denote that myoepithelial sialadenitis is associated with various autoimmune diseases (Bloch et al., 1965, Bertram, Feldkamp & Rossum, 1968, Pincus & [illegible] 1970, Talal et al., 1970) and the term immune sialadenopathy has been prop[illegible] (Nenci & Pellegrini, 1968).

There are only few reports on the [illegible]structure of normal human salivary glands [illegible] ner & Gansler 1961, Tandler 1962, [illegible] 1967, Tandler et al., 1970), but [illegible] munications have appeared that deal with [illegible] electron microscopic picture of salivary tumours. However, to the best of our know[illegible] there is only one previous report on the [illegible] structure of benign lymphoepithelial le[illegible] (Yarington & Zagibe 1969). Because of [illegible] it was thought worthwhile to present our [illegible] structural findings in a case of myoepith[illegible] sialadenitis.

RESULTS

Clinical findings

A 71 year-old woman with a past history [illegible] non toxic nodular goitre for which she [illegible] operated upon in 1948. She receives dig[illegible] and diuretics for the treatment of a m[illegible] arterial hypertension and a cardiosclerosis. [illegible] several years she has been complaining of [illegible] vere dryness in the mouth, but not in the e[illegible] Joint pains have not been present.

In 1968 an indolent tumour was recogni[illegible] in the left parotid region. It grew slowly [illegible]

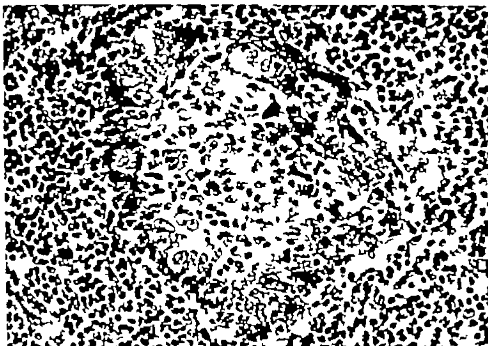


Fig. 1. Photomicrograph of benign lymphoepithelial lesion in the left parotid gland of a 71-year-old woman showing an epithelial island principally composed of central, light and peripheral, dark cells. Lymphoid cells occur both between the epithelial cells and around the island. ×100.

the following year and in January 1970 she was admitted. The cytological findings in material obtained at fine needle aspiration biopsy included degenerate cells among which were identified those of epithelial and lymphoid nature. Although no definite diagnosis could be presented, there was a suspicion of salivary gland neoplasm and an exploration was advised. Left-sided superficial parotidectomy was performed on January 20, 1970. Light microscopic examination of the surgical specimen showed a benign lymphoepithelial lesion (see below).

The postoperative course was uneventful and the patient was discharged. Soon thereafter she was readmitted in order to find out whether there was a Sjögren's syndrome, a diagnosis which was overlooked at the first examination in 1970. She was found to have a typical keratoconjunctivitis sicca with conjunctival and corneal epithelial lesions stainable with Rose Bengal. Schirmer's test showed no lachrymal secretion and gustatory stimulation with citric acid disclosed complete lack of secretion from the major salivary glands. Sialography was not possible because of her xerostomia. Laboratory findings included: ESR 31–50 mm/h, negative AST and ASTA, and positive acrylic test, hemagglutination test, and CRP. A paper electrophoresis showed elevated immunoglobulins (2.8 g/100 ml). There were no L.E. cells or antibodies directed against salivary gland cells.

Light microscopic findings

The basic lobular architecture of the gland was preserved but the parenchyma was almost totally replaced by lymphoid tissue and virtually no unaffected acini were seen. Most characteristic was a moderate number of ducts that were diffusely scattered in the lymphoid tissue, often in association with epithelial cell nests (Fig. 1). The ducts were lined by single-layered, usually cuboidal cells with light cytoplasm. Buds of epithelial cells were seen close to the ducts, and from these buds larger cell nests (islands) seemed to have been developed. Oc-

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ULTRASTRUCTURAL FINDINGS IN A CASE OF BENIGN LYMPHOEPITHELIAL LESION (SJÖGREN'S SYNDROME)

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(Received May 22, 1970)

Abstract A 71-year-old woman was admitted because of a slowly growing tumour in the left parotid region. Left-sided superficial parotidectomy was performed and light microscopic study of the surgical specimen disclosed benign lymphoepithelial lesion. The clinical and laboratory examinations showed a sicca syndrome that is a variant of Sjögren syndrome Ultrastructural investigation of the resected material confirmed the light microscopic finding of a salivary gland architecture that was destroyed and replaced by lymphoid tissue, cell islands and seemingly proliferating ducts. The duct cells, as well as some of the cells constituting the islands, were characterized by a cytoplasm f low density rather inconspicuous organelles and occasional delicate irregular filaments. Other island cells exhibited more electron dense cytoplasm, tonofilaments and prominent desmosomes. In addition, there were cells in the islands that possessed atures intermediate between those of the two other of cells. No indeterminate myoepithelial cells found. Although conclusive evidence is lacking, it is believed that the cell islands might be formed by proliferation and squamous cell metaplasia of duct cells.

The light microscopic alterations in the salivary glands are identical at Sjögren s syndrome, Mikulicz s disease and benign lymphoepithelial lesion (Godwin, 1952 Morgan, 1954 Seifert & Geiler, 1957 Grage & Lober 1964 Bark & Perzik, 1968) Proliferations of the ducts with the appearance of myoepithelial cell islands have been suggested to be pathognomonic for these conditions that often are referred to as myoepithelial sialadenitis (Seifert, 1966 Ericson, 1968 Ericson & Sundmark 1970) Recent investigations denote that myoepithelial sialadenitis is associated with various autoimmune diseases (Bloch et al., 1965 Bertram 1967 Feldkamp & Rossum 1968 Pincus & Dekker 1970 Talal et al., 1970) and the term autoimmune sialadenopathy has been proposed (Nenci & Pellegrini 1968)

There are only few reports on the ultrastructure of normal human salivary glands (Ferner & Gansler 1961 Tandler 1962 Garrett, 1967 Tandler et al 1970) but numerous communications have appeared that deal with the electron microscopic picture of salivary gland tumours. However to the best of our knowledge, there is only one previous report on the fi structure of benign lymphoepithelial lesion (Yarington & Zaglbe 1969) Because of this it was thought worthwhile to present our ultrastructural findings in a case of myoepithelial sialadenitis

RESULTS

Clinical findings

A 71 year-old woman with a past history of non-toxic nodular goitre for which she was operated upon in 1948 She receives digitalis and diuretics for the treatment of a moderate arterial hypertension and a cardiosclerosis. For several years she has been complaining of severe dryness in the mouth, but not in the eyes. Joint pains have not been present.

In 1968 an indolent tumour was recognized in the left parotid region. It grew slowly during

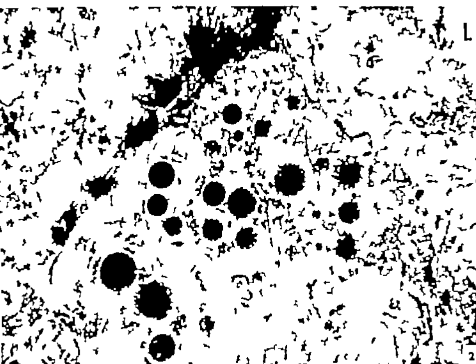
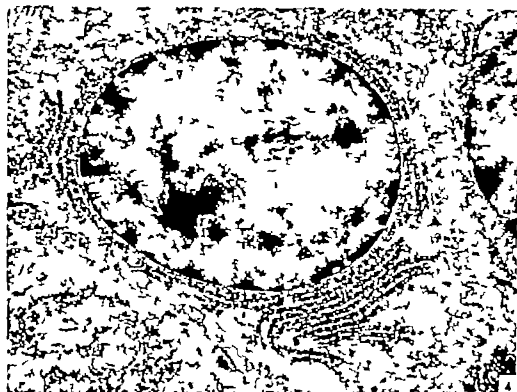




Fig. 2. Electron micrograph of portion of epithelium and lumen (*L*) of duct in benign lymphoepithelial lesion demonstrating round electron-dense granules, delicate cytoplasmic filaments, and several desmosomes. 30 000.

Fig. 3. Portion of epithelial island in benign lymphoepithelial lesion showing cell with low cytoplasmic electron density, free ribosomes, vesicles of endoplasmic reticulum, centriole and some mitochondria. 31 000



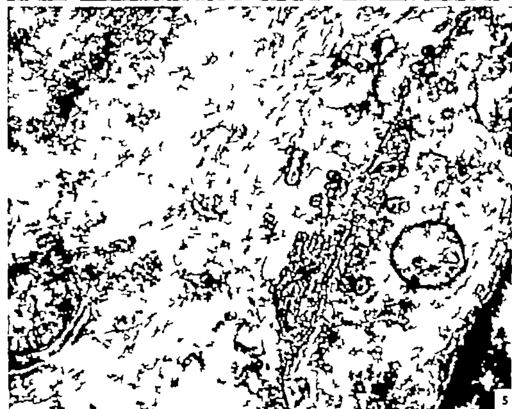


Fig 4 Other cells in epithe wing low cytopl ron density a nular endo lum. 16 0

Fig 5 Island cells showing cytoplasm of low densit that contains delicate filaments in irregular arrange ment. *Mitochondria*, endoplasmic reticulum and smal vesicles are also seen. 47 000.





Fig 6. Portion of island showing cells (*D*) that mainly are characterized by moderate cytoplasmic electron density and tonofilaments (*f*), as well as cells with less dense cytoplasm (*L*). × 8 000.

Fig 7. Island cell showing moderate cytoplasmic electron density, tonofilaments, a desmosome, mitochondria and ribosomes. × 42 000



Fig 8 Island cells with low cytoplasmic electron density that are surrounded by electron-dense membranes of varying thickness. 10 000

Fig 9 Higher magnification of membranous stru tures showing cross-striated subunits, probably r presenting collagen. 18 000

cate filaments. These were interpreted as tonofilaments. Numerous well developed desmosomes were found in the dark cells.

There were also some cells which appeared to be intermediate between the light and the dark cells. Some of them contained both del cate filaments and tonofilaments. There we no cells in those islands that possessed all tl characteristics of myoepithelial cells.

Electron-dense membranous structures we



Fig. 10 Epithelial island composed f cells with or without tonofilaments (*f*). Between the pithelial cells there are small dense lymphoid cells, one of them (*L*) penetrating the electron-dense membra rounds the island. 5 000.

often observed around the islands (Fig. 8) They were of varying thickness and were sometimes duplicated or multilayered. Similar structures were occasionally seen within the islands. They were, at least partly composed of collagen fibres (Fig. 9). Between the epithelial cells in the islands there were lymphoid cells (Fig. 10). The latter type of cell was also found to pene trate the membranous structures. No obvious lymphoid cells were observed intracellularly in the epithelial cells.

DISCUSSION

The clinical and laboratory findings in the present case indicated a sicca syndrome (Bloch et al., 1965) The diagnosis was morphologically verified at light microscopic examination, which showed a picture conforming to ported in cases of Sjögren s syndrome licz's disease and benign lymphoepithel sion. Thus, lymphoid tissue, ducts, and epith cell nests were found. Such nests of cells we observed by Morgan & Castleman who in 1953 introduced the term epi-myoepithelial islands for alterations in the duct epithelium in a loca tion which normally would be accorded to myoepithelial cells" Although this designation has been extensively used in the subsequent litera ture the existence of myoepithelial cells in these islands has seldom been questioned However the identification of these cells presents a problem (Welsh & Meyer 1968 Tandler et al 1970) In general, myoepithelial cells occur around acini and intercalated ducts (Mylius 1960 Tamarin, 1966 Bogart, 1968 Shear 1969) They have been stated to be lacking

(Takahashi, 1958) or rare (Kawakatsu & Mori 1962 Tandler 1965 Garrett, 1967) in human striated ducts. Myoepithelial cells have been recorded in mixed tumours of the salivary glands (Mylius, 1960 David & Korth 1960 Doyle et al. 1968 Welsh & Meyer 1968) They have also been suggested to occur in adenoid cystic carcinoma (cylindroma) (Hübner et al. 1969 Hoshino & Yamamoto 1970) Markert (1965) however failed to show myoepithelial cells in this type of tumour

In the present case cytoplasmic filaments were found in cells both of the light and the dark variant. Some of these appeared to represent tonofilaments, whereas others were more delicate and irregularly arranged. Focal densities and parallel organization of the filaments that have been stated to characterize myoepithelial cells were not clearly observed. Other distinguishing features of non-neoplastic myoepithelial cells have been suggested to be a relative sparseness of cell organelles (Leeson & Jacoby 1959) presence of micropinocytotic vesicles (Tamarin, 1966), as well as a fairly frequent occurrence of nerve endings in direct or indirect contact with the cell (Garrett, 1967 Yamaguchi, 1967 Harrpo & Mackay 1968). ...oplastic myoepithelial cells, on the other ...d, have been stated to lack pinocytotic vesicles and associated nerve endings (Hoshino & Yamamoto, 1970)

The tonofilaments appeared most often in cells with prominent desmosomes and electron-dense cytoplasm These cells were similar to the epidermoid-type cells" (Welsh & Meyer 1968) and the "type B cell" (Kierzenbaum, 1968) that have been suggested to occur in salivary gland tumours. Tonofilaments have been found in salivary gland tumour cells by Eneroth & Wersäll (1966) Inasmuch as cytoplasmic tonofilaments occur in some cell components of squamous epithelium their occurrence in the present case might denote a metaplasia from secretory cells into squamous epithelium cells. Some evidence of metaplasia has been obtained also in other studies of pathologically altered salivary glands (Godwin, 1952 Eneroth & Wersäll, 1966 Font et al., 1967 Naji et al 1968 Hoshino & Yamamoto, 1970) In the exocrine pancreas which structurally is related to the parotid gland, metaplasia into goblet cells is known to occur in the duct epithelium as a result of cellular damage (Boquist, 1969 *a* *b*)

The light cells in the present case bear some resemblance to the "clear cells" that have been observed in rat salivary glands and been believed to represent a transition stage between epithelial and "myoid cells" (Parks, 1961) In the human submandibular gland a transformation of clear cells to myoepithelial cells has also been proposed by Tandler (1965) Mylius (1960) could not exclude the possibility of intermediate forms between myoepithelial cells and secretory cells in mixed tumours. Although the light cells might be of intermediate nature, no evidence was obtained of an evolution into myoepithelial cells. There were also some seemingly intermediate cells which possessed both delicate filaments and tonofilaments These findings may indicate the existence of intermediate stages in a development of duct cells to squamous epithelium cells. The light cells were also somewhat similar to the centro-acinar ductule, and agranular cells that have been thought to be involved in new formation or regeneration of endocrine cells in the pancreas (Boquist & Falkmer 1970).

Yarington & Zagibe (1969) believe that the benign lymphoepithelial lesions arise from embryonic nests in the glands rather than from ductule metaplasia or "epithelioid hyperplasia in lymphoid tissue The present findings seem to denote that the epithelial cell islands are derived from ducts by a process of proliferation and squamous metaplasia of duct epithelium.

The interstitial substance that is present in some pathologically altered salivary glands has been suggested to be formed by secretion from tumour cells (Azzopardi & Smith, 1959 Quintarelli & Robinson, 1967 Eneroth et al., 1968) or from myoepithelial cells (Hübner et al., 1969). No conclusions can be drawn from the present study as to the origin of these mem-

branous structures, but at least some portions of them were of collagen nature.

The lymphoid cells occurred in the cell islands between the epithelial cells but not within their cytoplasm as reported by Marruzzi et al. (1966). Yarington & Zagibe (1969) concluded that the stimulus leading to hyperplasia of lymphoepithelial tissue "may be related to some unknown element within the chronically infected gland or be related to some form of autoimmune incident". On the basis of the present findings it is not possible to suggest anything with certainty concerning a possible pathogenetic mechanism and a possible relationship between epithelial and lymphoid cells. There is apparently need for further knowledge about cellular hypersensitivity and the cytotoxic effects of lymphoid cells in autoimmunity (Soborg & Bertram, 1968; Perlmann & Holm, 1969).

ZUSAMMENFASSUNG

Bei einer 71-jährigen Frau mit einem langsam wachsenden Parotistumor wurde eine linksseitige partielle Excision vorgenommen. Die lichtmikroskopische Untersuchung ergab gutartige, lymphoepitheliale Läsionen. Die klinischen und Laboratoriumsbefunde deuteten auf ein "Sicca-Syndrom", einer Variante des Sjögren-Syndroms, hin. Die elektronenmikroskopische Untersuchung bestätigte den lichtmikroskopischen Befund zerstörten Speicheldrüsengewebes sowie das Auftreten lymphoiden Gewebes, Zellinseln und wahrscheinlich proliferierenden Speicheldrüsengängen. Die Gangzellen und einige der Inselzellen waren gekennzeichnet von einem elektronendünnen Cytoplasma, ziemlich wenigen Zellorganellen und zerstreuten, dünnen, unregelmässigen Filamenten. Andere Zellen in den Inseln zeigten elektronendichtes Cytoplasma, Tonofibrillen und deutliche Desmosomen. Es gab auch Zellen mit intermediärem Aussehen. Kein Vorkommen sicherer myoepithelialer Zellen. Obwohl der Nachweis fehlt, nimmt man an, dass die Zellinseln mit Proliferation und Plattenepithelmetaplasie von den Gangzellen gebildet werden können.

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PRESBYACUSIS

IV Forward vs Reverse Frequency Sweep Audiometry

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Abstract. Sixty presbyacutic ears (30 subjects) were studied with forward and reverse sweep frequency technique between 125–8 000 Hz using Grason-Stadler Model E 800 audiometer The sweeps with continuous tones gave slightly better threshold values in forward than in reverse direction at middle and high frequencies, the situation being opposite at low frequencies. The starting direction (125 or 8 000 Hz) did not affect the results. For interrupted stimuli the thresholds were overlapping. None of the ears showed abnormal fatigability and the amplitude of threshold tracings suggested the absence of recruitment. Thus, degenerative changes in the end organ or in the ganglion cells due to presbyacusis do not cause abnormal separation of forward and reverse sweep frequency tracings.

In self-recording audiometry the threshold measurements are carried out with interrupted or continuous tones, using either fixed or sweep frequency technique, the latter starting from the low tones. Results obtained in presbyacusis with the former method have been presented earlier (Jokinen, 1969 1970 *a*, *b*)

Using the sweep frequency method, Corso & Wilson (1957) tested 10 normally hearing subjects, aged 17–25 years, utilizing reversed (descending frequency order) frequency sweeping. At frequencies higher than 1 000 Hz the threshold values were better for interrupted and continuous stimuli in ascending frequency sweep than in the descending direction. below 1 000 Hz the situation was reversed Epstein (1960), in testing 15 subjects with perceptive hearing impairments between 2 000–4 000 Hz, obtained poorer thresholds at 3 000 Hz with ascending than with descending sweep

The series of Rose (1962) included 47 patients (17–81 years) suffering from sensorineural deafness. Sweep frequency testing was made in both directions with the continuous and interrupted stimuli between 100–10 000 Hz in 7.5 min, with an attenuation rate of 2.5 dB/sec. If the hearing loss was 20 dB or less, the curves were generally overlapping. On patients with greater hearing losses, the high-to-low sweep with continuous stimuli gave poorer threshold values than other tracings.

T Palva et al. (1970) studied different types of sensorineural deafness and found abnormal reverse tracings in some cases. Their series consisted of 13 presbyacutic ears, extended to 22 in the report by Kärjä & A. Palva (1970) in all of these the forward and reverse Békésy tracings with continuous tone were overlapping. In the present study the tests were repeated and supplemented with a larger material.

MATERIAL AND METHODS

The material consisted of 30 patients with presbyacusis, ranging in age from 68 to 87 years, with an average of 75 years. Only 7

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Table I. *Comparison of forward and reverse sweep frequency thresholds for continuous and interrupted tone*

	Start from low tones				Start from high tones			
	Continuous tone		Interrupted tone		Continuous tone		Interrupted tone	
Frequency	T.D	S.D	T.D	S.D	T.D	S.D	T.D	S.D
125	1.9	4.0	1 1	3.3	0.5	3.4	0.2	3.4
250	0.1	3.2	0.3	2.9	0.2	3.1	−0.8	3.8
500	0.0	3.6	−0.5	2.6	−1 4	3 6	0 4	2.8
1 000	−1.6	3.2	0.6	2.9	−1.3	2.1	−1 4	3.8
2 000	−2.8	5.4	−1 0	4.1	−0.8	3.5	−0.1	2.8
3 000	−1.9	7.0	−0.1	5.4	1.[illegible]	11.2	−0.6	2.8
4 000	−1 7	4.8	−0.3	3.2	0.8	3.8	0.3	3.7
6 000	−1.3	5.5	0.5	3.4	−1 0	3.0	−0.2	3.5
8 000	−2.1	10.4	0.4	3.5	0.1	4.3	0.8	2.5

Negative sign indicates that the threshold obtained in high–low direction was poorer T.D = Difference of forward and reverse thresholds. Significance levels: $p < 0.05$ $p < 0.01$ $p < 0.001$

subjects were under 70 The patients were free from ear diseases other than presbyacusis. Both ears were tested. the thresholds were measured first with the usual descending–ascending manual technique (Madsen Model OB 60 audiometer) The sweep frequency tracings were registered with the Grason-Stadler Model E 800 self-recording audiometer In alternate cases the sweep-tests were started from the low frequencies and in the other cases from the high frequencies. the run in the opposite direction in each case followed immediately after the first sweep Both interrupted and continuous stimuli were used for frequencies 125–8 000 Hz, the intensity being varied at a rate of *4.2 dB*/sec. The sweep in one direction took 3 ²/₃ min. The pulses and pulse intervals were of 200 msec duration with a rise and fall time of 25 msec.

The test subjects were instructed to press the key as long as they heard the tone and release it immediately when the tone disappeared. If the forward and reversed tracings differed more than 10 dB two-octave frequency sweep technique was applied as control measurement. For thresholds, the midpoints of the tracings were determined and the average curves drawn through these points. In addition, threshold tone decay was measured during one minute at *1 000* and *4 000 Hz* with *fixed frequency technique Amplitudes of* threshold tracings were determined, interpolating the excursion maxima and minima Statistical treatment of the data took place at the Computer Centre of the University of Oulu, and the Student's *t* test was used to determine the significance levels.

RESULTS

Table I shows the *forward* and *reverse* frequency sweep threshold differences for interrupted and continuous tones. Starting from low frequencies (Fig. 1) the thresholds for continuous tones tended to be poorer in reverse testing order above *500 Hz*, whereas the forward values were poorer below it. However the greatest differences at 125 1 000 and 2 000 Hz were only statistically suggestive ($p < 0\ 05$) In one case only of 15 subjects, was there a separation amounting to 20 dB for 4 000–8 000 Hz in a control test with the two octave technique even these tracings were overlapping. In other cases the separation varied between 0–8 dB

A similar picture emerged when continuous tone testing was started from the high frequencies (Fig. 1) The difference became significant ($p < 0\ 01$) for 1 000 Hz, and was suggestive *for 500 Hz* ($p < 0\ 05$) *Individual dif*

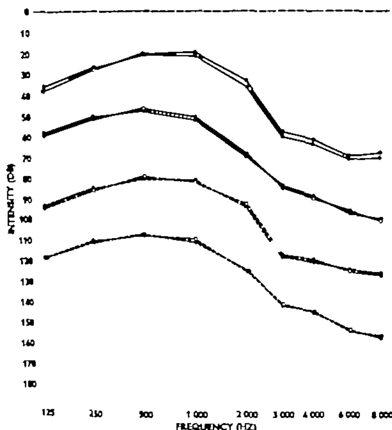


Fig 1 Forward and reverse tracings for continuous (——) and interrupted ( ) tones in presbyacusis. Each pair of curves are means for 15 ears. Open circles represent forward and closed circles the reverse frequency sweeps. In both test variations, the upper pair was registered starting from 125 Hz and the lower from 8 000 Hz. The three lower pairs of curves are artificially separated from the upper by 30 60 and 90 dB.

ferences between threshold values varied between 0–8 dB

For pulsed stimuli (Fig. 1) the tracings were overlapping irrespective of starting direction. The threshold values with pulsed stimuli were slightly better than those with continuous tone (Table II). Starting from the lowest frequency in the low-high direction the two tracings differed suggestively at 6 000 Hz and in the reverse sweep at 4 000 and 8 000 ($p \leq 0.05$) and in addition significantly at 6 000 Hz ($p \leq 0.01$)

When the tracings were started from the high end the difference for pulsed and continuous tracings became more evident. In high–low direction the pulsed tracings were statistically better at 2 000 4 000 and 8 000 Hz ($p \leq 0.01$) and suggestively better at 6 000 Hz ($p \leq 0.05$) In the forward run the interrupted tracing was significantly better at 3 000 and 4 000 Hz ($p \leq 0.01$) and suggestively better at 2 000 and 8 000 Hz ($p \leq 0.05$)

The excursion widths of threshold sweeps for both interrupted and continuous tones had a tendency to become narrower with increasing frequency though not statistically. *Table III* analyses the mean amplitude values at various frequencies when testing was started from the lowest frequency The excursion widths for continuous tones tended to be smaller than for interrupted stimuli at each frequency but no significant differences were found. The amplitudes between forward and reverse sweeps differed statistically for neither interrupted nor continuous tones. The behaviour of results was the same when recording was started at 8 000 Hz.

Threshold tone decay measured at 1 000 Hz was below 5 dB in 52 ears and between

Table II. *Comparison of sweep frequency thresholds for interrupted and continuous tones*

	Start from low tones				Start from high tones			
	Forward		Reverse		Forward		Reverse	
Frequency	T.D.	S.D	T.D	S.D	T.D	S.D	T.D	S.D
125	4.0	11.3	3.2	11.9	1.1	10.1	0.7	9.6
250	2.8	14.3	3.0	15.0	1.2	7.4	0.5	8.4
500	1.1	9.8	0.5	9.3	−0.5	7.0	1.3	7.5
1 000	−1.5	7.4	0.6	6.6	1.6	7.6	1.4	7.8
2 000	1.1	10.8	2.9	8.1	4.4	10.3	5.1	9.2
3 000	0.8	8.8	2.5	9.5	4.6	8.6	2.8	16.0
4 000	2.1	8.7	3.6	7.0	5.3	7.2	4.8	8.9
6 000	4.8	11.5	6.6	11.9	3.3	9.1	4.1	8.6
8 000	1.1	10.1	3.6	8.6	3.6	8.0	4.3	7.5

Negative sign indicates that the threshold obtained by interrupted tone was poorer than by continuous tone.
T.D. = Threshold difference of pulsed and continuous tones. Significance levels: $p < 0.05$, $p < 0.01$, $p < 0.001$

6–10 dB in 8 ears, at 4 000 Hz in 49 and 11 ears, respectively.

The left and right ears behaved similarly on all test occasions.

DISCUSSION

The forward sweep for continuous stimuli gave slightly better threshold values than the reverse run at the middle and high frequencies, and the reverse tracing at the low frequencies. These results are in agreement with those of Corso & Wilson (1957) and T. Palva et al. (1970). The difference was significant at 1 000 Hz and suggestive at 125, 500 and 2 000 Hz. For interrupted sweeps there was no separation of the curves.

Thus, all test occasions had the feature in common that, if the threshold tracing became better in relation to zero level, the tracing in that direction always ran below the tracing in opposite direction in the same region. This is probably due to the registering technique used in Békésy audiometry. In approaching the descending threshold from above and correspondingly the rising threshold from below, the tracing registered with the tracking intensity method lags behind.

The interrupted tracings gave slightly better thresholds than the continuous tones and the differences were significant at higher fre-

Table III. *Average excursion widths for interrupted and continuous tone, start from low tones*

	Continuous tone				Interrupted tone			
	Forward		Reverse		Forward		Reverse	
Frequency	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D
125	16.8	5.1	17.2	5.3	18.6	4.9	19.7	6.4
250	17.2	5.7	17.1	7.4	17.9	5.3	18.9	5.4
500	17.9	5.8	16.2	4.7	17.9	5.5	18.1	6.1
1 000	17.5	7.8	17.3	5.5	18.7	5.6	17.7	5.5
2 000	16.5	5.2	19.0	9.4	19.4	7.0	18.0	9.8
3 000	17.5	6.5	15.5	6.5	18.6	6.3	16.7	8.8
4 000	15.2	5.3	14.4	5.5	19.2	7.3	16.0	6.0
6 000	17.0	6.9	15.3	5.2	18.8	7.0	16.5	6.1
8 000	14.4	6.3	14.8	7.2	18.2	6.8	16.4	5.6

quencies (Table II). This is in accordance with the results published earlier (Jokinen, 1969). It should be noted that the starting point, at the low or high end, did not affect the results.

Rose (1962) and T. Palva et al. (1970) described patients with sensorineural deafness who showed an abnormally poor reverse tracing for continuous stimulus. This separation was associated with pronounced threshold tone decay and generally with absent loudness recruitment. In this study there was only one subject in whom the reverse continuous tone sweep gave a threshold 20 dB poorer than obtained with the forward curve at the frequencies 4 000–8 000 Hz. This may have been due to the patient's inexperience with the recording technique, since it did not appear in the control experiment using a two-octave run. Her threshold tone decay during 1 min was less than 5 dB for 1 000 and 4 000 Hz.

None of the 60 presbyacutic ears showed abnormal threshold adaptation values at the frequencies whereas the amplitude of the threshold tracings as a rule exceeded 5 dB, suggesting the absence of recruitment (T. Palva, 1957). It thus seems that the underlying pathology, i.e. vascular changes in the stria vascularis and end organ or ganglion cell degeneration, contribute neither to abnormal fatigability nor to poor reverse frequency sweep tracings, which are associated with more central auditory pathways (T. Palva et al., 1970).

ZUSAMMENFASSUNG

Sechzig altersschwerhörige Ohren (30 Subjekte) wurden unter Anwendung des Grason-Stadler E 800 Audiometers mit Vorwärts- und Rückwärtsstreichfrequenztechnik zwischen 125–8 000 Hz untersucht. Die Dauertonkurven ergaben bei mittleren und höheren Frequenzen bei Vorwärtsrichtung etwas bessere Gehörschwellenwerte als bei Rückwärtsrichtung. Bei niedrigen Frequenzen war die Lage umgekehrt. Bei Impulstönen überschnitten sich die Gehörschwellen. Der Ausgangspunkt (125 oder 8 000 Hz) hatte keinen Einfluss auf die Resultate. Kein einziges Ohr zeigte Spuren von abnormaler Ermüdung und die Amplitude der Gehörschwellenkurven zeigte das Nichtauftreten des Ausgleichseffekts („Recruitment") an. Die altersschwerhörigen pathologischen Veränderungen im Endorgan oder die Degeneration der Ganglienzelle bewirkten keine abnormale Separation von den Vorwärts- oder Rückwärtsstreichfrequenzkurven.

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PRESBYACUSIS

V Filtered Speech Test

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Abstract The effect of age on discrimination of filtered speech was studied with two different frequency bands using the monaural test and Matzker's binaural test. The intelligibility of filtered speech fell very early as a function of age. In the binaural test deterioration was noted in the group 30–39 years, taking the group 20–29 years as basis of reference. The change in intelligibility occurred before any changes appeared in pure tone thresholds in the frequency area of the filtered speech test. After 60 years, a significant asymmetry appeared in discrimination of filtered speech. Intelligibility was better in the binaural test and the monaural test of the left ears than in the right ears. This difference was attributed to the effect of cerebral dominance becoming evident in the degenerated central auditory pathways. Under 60 years of age the compensatory mechanism of the centrencephalic system apparently prevents the effect of cerebral dominance in filtered speech test.

Most audiological studies on presbyacusis deal with pure tone audiometry Several parameters of pure tone tests were reported in the four previous articles in the present series of audiological analyses of presbyacusis (Jokinen, 1969 1970 *a b* Jokinen & Kärjä, 1970) The pure tone audiometrical results in young patients are known to differ in some respects from those obtained for old people. In addition, it has been well established clinically that speech discrimination is more affected in the aged than in the young (Gaeth, 1948 Cawthorne, 1951 T Palva, 1952 Pestalozza & Shore, 1955 Goetzinger et al., 1961 Klotz & Kilbane, 1962 König, 1969) T Palva (1952) found discrimination losses of 3 to 20% in cases of presbyacusis studied with Finnish speech test material Pestalozza & Shore (1955), using PB words, reported that speech discrimination was always 9 to 20% better in the young than in old people with the same amount of pure tone hearing loss. This phonemic regression" (Gaeth, 1948) has been explained by a diminished integrative capacity of the aged (Calvi & Finzi, 1957) In difficult listening conditions this reduced discrimination of speech becomes more evident. Bordley & Haskins (1955) Finzi (1956) and Calearo & Lazzaroni (1957) found poor discrimination with accelerated speech in presbyacusis similar to that in cortical lesions. The same finding was obtained by interruption of speech (Bocca, 1956, 1958 Antonelli et al., 1963 Kirikae et al., 1964) Kirikae et al., (1964) distorted the words with a low pass filter (1 200 Hz) and found in 10 subjects with presbyacusis (age from 50 to 70 years) discrimination of about 25% at sensation levels of 40–60 dB. The corresponding figure in a group of young people was about 40% No comparison between the left and the right ear was made

The binaural speech audiometric methods specially devised for detection of central hearing disorders show in presbyacusis, changes, which are much like those in diffuse lesions of the central nervous system. In the switched speech test, Kirikae et al. (1964) found a lowering of discrimination comparable to the findings with the interrupted speech.

This study was supported by grant from the National Council for Medical Sciences.

Inglis (1962) and Inglis & Ankus (1965) used the dichotic speech test developed by Broadbent (1954), who presented different words simultaneously to different ears. They found that discrimination decreases continuously with age Feldman (1965 1967) using in principle the same test, stated that discrimination falls markedly after 70 years. He did not find differences between the right and the left ear The age related impairment shown in this test was thought to be due to a decline in the efficiency of short-term memory storage process, not in auditory perception as such (Inglis, 1965 Inglis & Ankus, 1965)

Matzker's binaural test (1958) is based on the principle where the different filtered speech bands are presented one to each ear The central synthesis and integration makes this speech intelligible though each band separately is not intelligible This test was found to be positive in the age group 60–64 years in 15% and in the group 65–74 years in as many as 82.4% Discrimination in this test began to decrease markedly after 40 years age (Beyermann-Grösser 1959) In Matzker's opinion a positive test indicates a lesion in the brain stem area. Hayashi's (1965) series of 116 patients with various hearing disorders tested by Matzker's method or by Hayashi's modification which consisted also of a monaural filtered speech test, included 7 cases of presbyacusis aged from 55 to 76 years. They all showed normal test results.

A. Palva (1965) combined the monaural filtered speech test and Matzker's binaural filtered speech test to obtain a practical test for routine clinical use. This test has now been used to study the possible changes in discrimination of filtered speech in presbyacusis, using people of young age groups as control material.

MATERIAL AND METHODS

The study consisted of 149 test subjects, 106 women and 43 men, ranging in age from 20 to 89 years. A group of 20 healthy people, 20 to 29 years of age (mean 22 years) formed the main reference group There were also 20 subjects in each of the age groups 30–39 years, 40–49 years and 50–59 years further 34 subjects in the group 60–69 years, 20 in the group 70–79 years and 15 in the group 80–89 years. Mean ages in these groups were 34 45 55 64 74 and 85 years, respectively Six test subjects were left-handed.

None of the test subjects had any ear disease other than possible presbyacusis and there was no history of noise exposure The routine clinical neurological examination gave negative findings in each case.

Pure tone threshold measurements by air and bone conduction were made at 125 250 500, 1 000 2 000 4 000, 6 000 and 8 000 Hz with a Madsen Model 60 audiometer equipped with Beltone TDH 39 earphones. If there was a marked (> 10 dB) air-bone gap no further tests were made. Calibration of the audiometer was made according to the ISO-standard (Davis & Kranz, 1964)

Undistorted speech audiometry was carried out by the method of T Palva (1952), measuring speech reception threshold (SRT) and discrimination of speech 30 dB above the SRT In this test a Madsen Model SU 20 speech unit was connected to the system used in pure tone audiometry

In the filtered speech test the same equipment was used as in normal speech audiometry except that, instead of TDH 39 earphones, insert type M 81 earphones were employed.

The filtered speech test was carried out by the method developed by A Palva (1965) The basic principles of this test are as follows. the necessary frequency distortion is achieved by using two separate bands filtered from speech. In this study bands 480–720 Hz and 1 800–2 400 Hz were used. These are shown in Fig. 1 The former band gives alone a discrimination score of 17% the latter 15% The whole test is carried out at 50 dB sensation level which is made possible by eliminating the cross-hearing with insert-type earphones. The subject hears on both bands the first test word

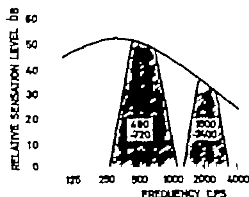


Fig 1 Bands used in filtered speech test. The top curve shows the ideal shape of the speech spectrum.

in his right ear and the next word in his left ear The third test word comes to one ear on the low band and to the other ear on the high band, as in Matzker's test. The fourth word comes in the same way as the first, and so on. The words follow each other at 5 sec intervals. Each of the three test objects, the right ear the left ear and binaural resynthesis test are measured by each of the three 30-word lists of test words specially selected for this purpose The test words had been recorded with a two-channel tape-recorder ready filtered, so that when the channels are coupled to a two-channel audiometer and fed into earphones, the test conditions change automatically in the way described above. Three discrimination scores are obtained two refer to the monaural filtered speech test with the right and the left ear the third to the binaural hearing resynthesis test. Each score is calculated on the basis of 90 test words Since the test object changes after each word, all three parts of the test are mutually comparable and equally valuable

The statistical analyses were performed in the Computer Centre of the University of Oulu. The averages and their standard deviations were calculated. *Comparison of two averages* were made by applying the Student's *t*-test' the difference is termed significant if the corresponding value of probability (p) is $< 0\ 01$

RESULTS

The pure tone thresholds are shown in Table I The averages for both ears were calculated separately in each age group and at each frequency No significant difference between the

Table I. *Average pure tone thresholds in different age groups*

Age Group		Frequency (Hz) 125		250		500		1 000		2 000		4 000		6 000		8 000	
		Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S D	Mean	S.D	Mean	S.D	Mean	S.D
20–29	Right	13 7	5 4	8.2	4.8	6.0	4.6	3.8	3.8	2.0	4.0	5.8	4.8	5 7	4.9	6.5	7 6
	Left	13.5	5.5	10.8	4.0	6.2	4 7	4.5	3.5	3.5	3.9	5.2	7 0	6 0	6.6	8.2	8.1
30–39	Right	13.8	5.0	9.5	4 4	7.0	4.0	5.0	4.2	4.2	5.3	7.8	5 4	8.1	8.9	7.2	9.3
	Left	10.5	5.3	12.5	4.0	9.0	4 1	7.5	3 0	6 0	6.2	9.2	5.8	10.8	7.3	10.5	10.6
40–49	Right	15.0	6.5	12.0	5.8	8.2	6.6	7.8	5 4	8.5	7.9	19.2	17 1	17.5	17.5	16.8	14.3
	Left	15.5	6.7	12.8	5.6	10.0	5.9	9.8	5 4	8.5	8.2	18.5	12.6	18.2	12.3	19.5	17 4
50–59	Right	14.0	4.9	10.8	4.5	8.2	4.5	9.8	7 7	12.2	10.5	27.8	19.3	24.5	19.9	24.0	17 6
	Left	16.2	5 4	14.0	5.1	10.8	6.2	11 0*	7.2	13.5	12.6	28.2	16.1	27 1	19 1	27.0	19.8
60–69	Right	20 6	9 1	17.2	9.2	16.3	8.5	16 6	9 1	25 7*	14.9	42.2	21.5	44 7	19.9	51 6	23.3
	Left	21.2	10 4	17.2	10.1	17.9	9 6	18.2	9.8	25 7	15.5	45.0*	19 4	46.8	20.3	52.9*	21 7
70–79	Right	21.2	7 0	17.0	6.8	16.2	7.0	20.0	7 4	27.0	8 6	42.0*	19 0	47.2	19.0	58.2	16 2
	Left	20.5	6.3	17.0	6.2	17.5	6.4	20.8	6.9	25.5	9 7	47.5	16.4	51.5	17.2	58.2	17.9
80–89	Right	24.0	7 8	21.0	6.9	21 7	6.4	22.3	8.4	30.0*	8.2	45.0	11.8	55.0	13.1	67.0	15.2
	Left	25 7	7.5	24 7	6.9	23.7	7.2	21 7	8.4	28.3	8.8	49 7	11 1	56.7	13.3	72.3	11.8

Differences have been calculated relative to the youngest age group. Significance level: $p < 0.01$

Table II. *Discrimination scores in undistorted speech audiometry*

	Age group													
	20–29		30–39		40–49		50–59		60–69		70–79		80–89	
	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D
Right	97.6	2.6	97.9	2.2	96.4	4.3	96.2	3.8	92.1	5.6	87.7	7.7	87.8	6.4
Left	97.6	3.0	97.2	3.1	97.1	2.8	95.6	3.7	92.7*	5.6	87.1	10.6	85.6	5.6

Differences have been calculated relative to the youngest age group.
Significance level, $p < 0.01$

right and the left ear was obtained for any frequency or age group. The first significant alterations in thresholds due to presbyacusis appeared in the age group 40–49 years at 2 000, 4 000 and 8 000 Hz. The group 60–69 differed significantly from the youngest group for every frequency studied, and this applies also to groups 70–79 and 80–89 years.

The speech audiometric data are demonstrated in Table II. Discrimination of undistorted speech in the three oldest age groups was significantly poorer than in the youngest group. However discrimination ability was quite high even in the oldest group, showing a loss of no more than 12–24%. There was no significant difference between the right and the left ear in any one of the age groups.

Results of the filtered speech test are illustrated in detail in Fig. 2. The corresponding mean values and their standard deviations are shown in Table III. A significant difference between the first and the second age groups was already noticed in the binaural test, where the discrimination values were 90.2±4.9 and 83.7±7.2% respectively. Discrimination was found to fall further with an increase in age. The difference in discrimination between the groups 20–29 and 40–49 was over 10% and

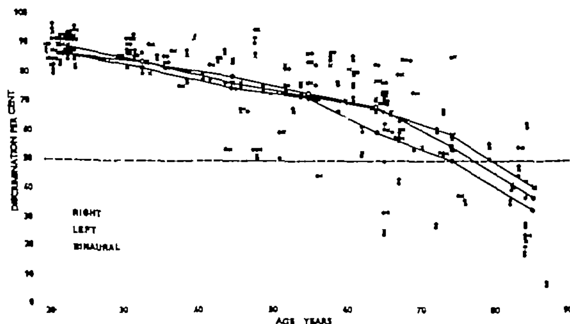


Fig. 2. Effect of age on filtered speech test.

Table III. *Discrimination scores in filtered speech test*

	Age group 20–29		30–39		40–49		50–59		60–69		70–79		80–89	
	Mean	S.D	Mean	S.D	Mean	S.D.	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D
Right	87.4	5.2	82.0	8.2	75.2	13.8	71.6	12.2	60.3	16.1	50.4	19.0	32.7	15.2
Left	87.5	5.4	83.8	7.8	79.0	13.6	73.4	11.5	68.9	16.3	54.8	18.6	37.0	16.1
Binaural	90.2	4.9	83.7	7.2	77.0	13.0	72.6	11.4	68.8	15.8	59.4	18.4	41.1	17.4

Differences have been calculated relative to the youngest age group.
Significance level: $p < 0.01$

significant in both the right ear and the binaural test.

Starting from the group 50–59 all age groups differed significantly from the 20–29 group, in respect of all three test objects. There was a gradual further loss of discrimination with increasing age. Thus, in the oldest age group, on an average more than 50% of the test words were lost in the binaural and more than 60% in the monaural conditions.

Individual variations in results increased with increasing age (Fig. 2) This became evident in the group 60–70 years, where the range of discrimination percentage varied from 20 to 90 In all three oldest groups, there were poor discrimination values of about 20% In oldest age group no test result over 70% obtained.

Comparison of the results in regard to the three test objects showed no difference between right-ear left-ear and binaural hearing in the four youngest age groups In the remaining three groups, average discrimination of the right ear was poorer than that of the left ear and than the binaural test result. In none of the age groups, however was this difference significant owing to the wide variation of individual discrimination results.

To discover the real difference between left ear right-ear and binaural hearing the differences in discrimination were calculated in each individual case and the results treated statistically The results of this procedure are presented in Table IV Discrimination in the left ear was significantly better than in the right ear in the groups 40–49 years ($t = 3.5$ p 0.01), 60–69 years ($t = 6.8$ $p < 0.001$) and 70–79 years ($t = 3.7$ $p < 0.01$) Binaural discrimination was significantly better than that of the right ear in the age groups 60–69 years ($t = 5.4$, $p < 0.001$) 70–79 years ($t = 4.4$ $p < 0.001$) and 80–89 years ($t = 3.7$ $p < 0.01$) Discrimina-

Table IV *Differences between monaural and binaural discrimination scores in filtered speech test*

	Age group 20–29		30–39		40–49		50–59		60–69		70–79		80–89	
	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D.	Mean	S.D.	Mean	S.D
Left ear superior to right	0.1	4.5	1.8	5.3	3.9	4.8	1.9	7.5	8.6	7.3	4.5	6.1	4.3	10.7
Binaural test superior to right	2.8	4.6	1.6	6.0	1.9	4.4	1.0	5.9	8.5	8.9	9.0	8.9	8.3	8.4
Binaural test superior to left	2.7	4.7	−0.1	4.7	−2.0	5.4	−0.9	6.7	−0.1	7.0	4.6	9.1	4.1	12.5

Differences have been calculated separately in each individual case correlated to the test object mentioned.
Significance level. $p < 0.01$

Table V *Discrimination scores in 27 cases with 15% or larger difference between monaural and binaural discrimination*

Age group	Case no	Undistorted speech discrimination, %		Filtered speech test discrimination, %		
		Right	Left	Right	Left	Binaural
50–59	1	100	100	*83*	67	71
	2	93	100	*75*	58	71
	3	97	90	74	*82*	66
60–69	4	87	97	67	*83*	78
	5	93	90	54	*74*	*74*
	6	83	97	20	*46*	40
	7	93	93	57	62	*76*
	8	100	100	67	76	*85*
	9	93	93	50	67	*70*
	10	83	77	60	*65*	48
	11	97	97	58	*87*	80
	12	100	100	57	73	*82*
	13	87	90	40	42	*61*
	14	87	87	52	63	*71*
	15	96	93	64	*80*	76
70–79	16	67	73	28	34	*46*
	17	87	80	64	76	*81*
	18	83	93	19	26	*40*
	19	87	93	29	35	*58*
	20	93	77	35	48	*55*
	21	90	90	41	34	*55*
	22	90	93	70	82	*90*
80–89	23	87	77	52	34	*66*
	24	90	87	32	38	*56*
	25	77	90	33	*55*	47
	26	77	73	18	24	*43*
	27	97	80	14	*35*	26

The highest discrimination value for filtered speech in each case is italicised.

tion of the left ear did not differ significantly from the binaural result in any age group studied.

The differences between the discrimination scores in the left and right ears and binaural test were in most cases not over 5% in the same person and reached 15% in none of the cases in the three youngest age groups. Differences exceeding 15% were obtained from the group 50–60 years upwards in the various groups as follows. 50–59 years 3 cases (15%), 60–69 years: 1? cases (35%) 70–79 years: 7 cases (35%) and 80–89 years. 5 cases (33%).

The speech audiometric results of these 27 cases (17 women, 10 men) are given in Table V They all were right-handed. The highest discrimination score with filtered speech was obtained in the binaural test in 16 cases (60%) The left ear had the best discrimination of filtered speech in 8 cases, the right ear in 2 cases only In one case, left-ear and binaural hearing were equal and 20% better than the right ear hearing.

The undistorted speech audiometric results and pure tone threshold curves were compared in each case to discover the cause of the asymmetric result in filtered speech test. A correlation between these three tests was found in three cases only (nos. 4 6 and 25) In case 4 the pure tone threshold dropped steeply from 10 dB at 1 000 Hz in both ears to 45 dB in the left and 55 dB in the right ear at 2 000 Hz, the threshold falling gradually further towards higher frequencies. This fact may explain the poorer discrimination in the right ear In cases 6 and 25 discrimination of speech was poorer in the right ear in both the undistorted and the filtered speech test though no asymmetry appeared in pure tone thresholds

DISCUSSION

The material used in this study accords well with earlier studies of presbyacusis based on pure tone and nondistorted speech audiometry (Leisti, 1949 T Palva, 1952 de la Rosée, *1953 Hinchcliffe 1959 Glorig & Nixon*, 1962 Goetzinger et al., 1961 Corso, 1963 Jatho 1969) taking into account that 70% of the test subjects were women A significant reduction in speech discrimination was noticed in the same age range, the seventh decade, where a significant lowering of pure tone thresholds extends over the whole frequency area.

In the filtered speech test, discrimination falls very early as a function of age. Even the group 30–39 years shows a significant deterioration of discrimination in binaural hearing as compared with the youngest group This change in discrimination of filtered speech oc-

Table III. *Discrimination scores in filtered speech test*

	Age group													
	20–29		30–39		40–49		50–59		60–69		70–79		80–89	
	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D
Right	87.4	5.2	82.0	8.2	75.2	13.8	71.6	12.2	60.3	16.1	50.4	19.0	32.7	15.2
Left	87.5	5.4	83.8	7.8	79.0	13.6	73.4	11.5	68.9	16.3	54.8	18.6	37.0*	16.1
Binaural	90.2	4.9	83.7*	7.2	77.0*	13.0	72.6	11.4	68.8	15.8	59.4	18.4	41.1	17.4

Differences have been calculated relative to the youngest age group.
Significance level: $p < 0.01$

significant in both the right ear and the binaural test.

Starting from the group 50–59 all age groups differed significantly from the 20–29 group, in respect of all three test objects. There was a gradual further loss of discrimination with increasing age. Thus, in the oldest age group, on an average more than 50% of the test words were lost in the binaural and more than 60% in the monaural conditions.

Individual variations in results increased with increasing age (Fig. 2) This became evident in the group 60–70 years, where the range of discrimination percentage varied from 20 to 90 In all three oldest groups, there were poor discrimination values of about 20% In e oldest age group no test result over 70% obtained.

Comparison of the results in regard to the three test objects showed no difference between right-ear left-ear and binaural hearing in the four youngest age groups. In the remaining three groups, average discrimination of the right ear was poorer than that of the left ear and than th binaural test result. In none of the age groups, however was this difference significant owing to the wide variation of individual discrimination results.

To discover the real difference between left ear right-ear and binaural hearing the differences in discrimination were calculated in each individual case and the results treated statistically The results of this procedure are presented in Table IV Discrimination in the left ear was significantly better than in the right ear in the groups 40–49 years ($t = 3.5$ $p < 0.01$) 60–69 years ($t = 6.8$ $p < 0.001$) and 70–79 years ($t = 3.7$ $p < 0.01$) Binaural discrimination was significantly better than that of the right ear in the age groups 60–69 years ($t = 5.4$, $p < 0.001$), 70–79 years ($t = 4.4$ $p < 0.001$) and 80–89 years ($t = 3.7$ $p < 0.01$) Discrimina-

Table IV *Differences between monaural and binaural discrimination scores in filtered speech test*

	Age group													
	20–29		30–39		40–49		50–59		60–69		70–79		80–89	
	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D.
Left ear superior to right	0.1	4.5	1.8	5.3	3.9*	4.8	1.9	7.5	8.6	7.3	4.5	6.1	4.3	10.7
Binaural test superior to right	2.8	4.6	1.6	6.0	1.9	4.4	1.0	5.9	8.5	8.9	9.0	8.9	8.3	8.4
Binaural test superior to left	2.7	4.7	−0.1	4.7	−2.0	5.4	−0.9	6.7	−0.1	7.0	4.6	9.1	4.1	12.5

Differences have been calculated separately in each individual case correlated to the test object mentioned.
Significance level. $p < 0.01$

Table V *Discrimination scores in 27 cases with 15% or larger difference between monaural and binaural discrimination*

Age group	Case no.	Undistorted speech discrimination, %		Filtered speech test discrimination, %		
		Right	Left	Right	Left	Binaural
50–59	1	100	100	*83*	67	71
	2	93	100	*75*	58	71
	3	97	90	74	*82*	66
60–69	4	87	97	67	*83*	78
	5	93	90	54	*74*	*74*
	6	83	97	20	*46*	40
	7	93	93	57	62	*76*
	8	100	100	67	76	*85*
	9	93	93	50	67	*70*
	10	83	77	60	*63*	48
	11	97	97	58	*87*	80
	12	100	100	57	73	*82*
	13	87	90	40	42	*61*
	14	87	87	52	63	*71*
	15	96	93	64	*80*	76
70–79	16	67	73	28	34	*46*
	17	87	80	64	76	*81*
	18	83	93	19	26	*40*
	19	87	93	29	35	*58*
	20	93	77	35	48	*55*
	21	90	90	41	34	*55*
	22	90	93	70	82	*90*
80–89	23	87	77	52	34	*66*
	24	90	87	32	38	*56*
	25	77	90	33	*55*	47
	26	77	73	18	24	*43*
	27	97	80	14	*35*	26

The highest discrimination value for filtered speech in each case is italicised.

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The differences between the discrimination scores in the left and right ears and binaural test were in most cases not over 5% in the same person and reached 15% in none of the cases in the three youngest age groups. Differences exceeding 15% were obtained from the group 50–60 years upwards. In the various groups as follows. 50–59 years. 3 cases (15%) 60–69 years: 12 cases (35%) 70–79 years: 7 cases (35%) and 80–89 years: 5 cases (33%)

The speech audiometric results of these 27 cases (17 women, 10 men) are given in Table V They all were right handed. The highest discrimination score with filtered speech was obtained in the binaural test in 16 cases (60%) The left ear had the best discrimination of filtered speech in 8 cases, the right ear in 2 cases only In one case, left-ear and binaural hearing were equal and 20% better than the right ear hearing.

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In the filtered speech test, discriminat falls very early as a function of age. E the group 30–39 years shows a significant de terioration of discrimination in binaural he ing as compared with the youngest group i change in discrimination of filtered

curs much earlier than any changes appear in pure tone thresholds in the frequency area tested by filtered speech.

The most interesting finding in the present study is the discovery of asymmetry in filtered speech test in the three oldest age groups. At ages over 60 years the degenerative changes in the auditory system have reached a uniform grade, wholly disregarding the presence of arteriosclerosis (Taniguchi et al., 1968) and they are a constant finding in the peripheral and central auditory apparatus (Saxén 1939 1952 Schuknecht, 1955) In general, the pathological changes should be symmetrical, and thus an asymmetric test re sult would indicate functional asymmetry in the auditory system at those levels where asymmetry can occur physiologically The asymmetry of the results for the left and the right ear seems to be related to the cerebral dominance of hearing. According to Penfield & Roberts (1959) the left cerebral hemisphere is dominant for the whole complex mechanism of speech regulation. On the other hand Milner (1962) and Shankweiler (1966) state that the right temporal lobe is of greater importance than the left for many aspects of auditory func ions excluding speech perception. The domin- of the right temporal lobe for perception nonverbal patterned auditory stimuli is weaker than the corresponding dominance of he left temporal lobe for perception of speech Shankweiler 1966) The presentation of peech on left handed people can be on either hemisphere or in some cases on both (Milner et al., 1964). This being so, it is clear that opinions differ concerning cerebral dominance in hearing in clinical material depending upon the testing method used.

Most studies concerned with physiological right or left ear superiority in pure tone thresholds or nondistorted monaural speech audiometric tests (e g. Corso, 1957 1963 Jerger et al., 1959 Palmer 1964) have revealed no significant difference Palmer (1964) however reported slight tendency to right ear superior ity in speech perception and Corso (1957) a tendency to left ear superiority for pure tones. Glorig et al. (1957) obtained significantly better right ear thresholds both for speech and for pure tones, particularly among males and for higher tones. While Goetzinger et al. (1961) reported a significantly better over-all mean for the right ear discrimination score with W 22 words lists, the lists of Rush Hughes showed *no significant difference between the left and* the right ear in presbyacusis. The right ear has been found more efficient in perception of simultaneous dichotically presented speech (Kimura, 1961 *b* Dirks, 1964) This right ear effect held only for verbal material (Kimura, 1963 1964) In accelerated speech test and distorted speech test Calearo & Antonelli (1963) found no difference indicating cerebral dominance This result was confirmed by Dirks (1964) using low-pass filtered speech.

Clinical findings on patients with temporal lobectomy have shown that verbal tests are more affected by the lesions of the left than of the right temporal lobe (Hellbrun, 1956 Meyer 1959 Balthazar et al., 1961 Kimura, 1961 *a b*) In Hellbrun s material this asymmetry was found only in patients with dysphasic symptoms. In perception of dichotically presented melodies the right temporal lobe seemed to be dominant (Kimura, 1964) and affection of this lobe results in a reduced capacity of the left ear for hearing brief melodies (Shankweiler 1966) In a few cases the dominance of speech perception was on the right side and then the tests of verbal intelligence in the left ear were more affected by a lesion of this hemisphere (Lansdell, 1962)

Bocca et al. (1955) and Calearo & Antonelli (1963) found no evidence of cerebral dominance affecting interrupted or distorted (low-pass filtered) voice tests in patients with temporal lobe lesions. This lack of asymmetry can be explained by the view of Penfield & Roberts (1959) that the common relay of both auditory cortices connecting them to the only speech area situated in the dominant hemisphere should be recorded as the centrencephalic system, which has equal relationship with both

cortical areas. The existence of such a compensatory mechanism is well confirmed by the finding that the impairment in discrimination for distorted words in the contralateral ear often disappears shortly after surgical removal of the affected temporal cortex (Bocca et al., 1955)

All the tests mentioned above are made either on normal test subjects, or on predominantly young patients. In our series of age related bilateral diffuse lesions of the cerebral pathways, sclerotic changes must be assumed to affect markedly both the cortical areas and the whole centrencephalic system, and thus reduce the ability to compensate the greater influence of left cortical damage in symmetrical lesions. In these cases a reduced discrimination of speech contralateral to the dominant hemisphere is found.

In the binaural part of the filtered speech test indicating central hearing resynthesis, discrimination does not differ significantly from that of the better ear Thus, the binaural synthesis of two different frequency bands is not affected by age. This is opposed to Matzker's (1958) opinion but accords with Lindén's (1960, 1964) finding that the ability to resynthesize speech in this test is reduced by the same amount as the intelligibility of speech filtered to the same bands and presented monaurally to one ear

The fact that discrimination in the binaural test was in many cases (Table IV) even better than the monaural scores indicates that two different bands of speech may interfere with each other when fed through a degenerated monaural hearing system. This effect is compensated by the central mechanism when each band comes from a different ear The high discrimination score in binaural hearing is in agreement with the studies of Chappell et al. (1963) They found that normally hearing adults showed approximately 20% better intelligibility scores for monosyllable words presented binaurally with a background of conversation than when these words were presented monaurally The effect of binaural loudness summation was excluded. In roughly similar conditions Jerger et al. (1961) found the binaural discrimination to be 10% superior to the monaural. This improvement is usually related to the ability of binaural systems to separate sounds spatially (Cherry & Bowles, 1960 Chappell et al., 1963), but there must be some additional factors affecting intelligibility in the present study

In monaural presentation the signal is mainly carried along the pathways crossing to the contralateral hemisphere (Tunturi, 1946 Rosenzweig, 1951) This electrophysiological finding has been confirmed by clinical observations on patients with temporal lobe lesions (Bocca et al., 1955 Goldstein et al., 1956 Calearo & Antonelli 1963 Hodgson, 1967) In binaural conditions, the different signals coming simultaneously from each ear may be expected to activate the whole auditory system in the brain stem. The signal then spreads more widely than the monaural one and finally finds its way in synthesized form to the highest auditory centres on both sides. Thus, discrimination in the binaural test can be on the same level as discrimination of the better ear

ZUSAMMENFASSUNG

Die altersbedingte Herabsetzung der Diskrimination der filtrierten Sprache in monauralem und in binauralem Test nach *Matzker* wurde *mittels* zweier differenter Frequenzbänder untersucht. Die Verständlichkeit der filtrierten Sprache wurde schon im Alter von 30–39 Jahren im Vergleich zur Altersgruppe von 20–29 Jahren herabgesetzt. Die Diskrimination wurde mit zunehmenden Alter schlechter in den monauralen und binauralen Testen, und erschien früher als die Senkung der Reintonschwellen in den Frequenzen des Tests mit filtrierter Sprache. Im Alter von über 60 Jahren wurde bedeutsame Asymmetrie in der Diskrimination der filtrierten Sprache entdeckt. Die Verständlichkeit im Binauraltest und im Monauraltest des linken Ohres war besser als die des rechten Ohres. Dieser Unterschied der Diskrimination ist mit der Frage der zerebralen Dominanz verbunden. Der Dominierungseffekt erscheint wahrscheinlich wegen der Degeneration des zentrencephalischen Systems, welches im Alter von unter 60 Jahren den Dominierungseffekt in der Diskrimination der filtrierten Sprache hemmen kann.

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DIETARY PREVENTION OF HEARING LOSS

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Abstract Many epidemiological studies around the world have demonstrated that in populations where the incidence of coronary heart disease is high the blood cholesterol levels tend to be high, and the intake of saturated fats is also usually high. This hypothesis was tested for a period of 5 years in Finland, *on two populations aged 40–59 years, in two mental* hospitals in which the usual high saturated fat diet was continued in one hospital and a substitution of polyunsaturated fats was markedly increased in the other At the end of this time the incidence of coronary changes was significantly less in the latter hospital. The hearing was significantly better in all frequencies. At the end of the 5 year period the diets in the two hospitals were reversed. Four years after the diet reversal the hearing in the now low-fat hospital was improved and the hearing in the now high-fat hospital was deteriorating. The incidence of coronary heart disease followed the same pattern. The Finnish investigators concluded that an important factor in the prevention of coronary heart disease y well be a low saturated fat diet. Our audiocal findings similarly indicate that such a diet ay well arrest, if not reverse, hearing loss.

Our initial study (Rosen et al 1962, 1964 Jansen et al 1964) of the hearing of the primitive Mabaan tribe in a noise-free atmosphere in the southeastern part of The Sudan led us to studies of the effect of aging on hearing, and, in several culturally varied populations to investigate the differences in dietary habits and the incidence of cardiovascular disease (Rosen et al., 1964 Rosen & Olin, 1965)

Many epidemiological studies around the world have demonstrated that in populations where the incidence of coronary heart disease is high the blood cholesterol levels tend to be high, and the intake of saturated fats is also usually high. From this evidence has emerged a hypothesis, according to which a high content of saturated fats in the diet disturbs the normal content and composition of the blood lipids. This change accelerates the development of atherosclerosis and thrombotic phenomena and may lead to increased incidence of coronary heart disease. If it is true that a high saturated fat diet is a prime factor in this development, could a reversal in diet show a similar or parallel change in the hearing? And therefore might not diet be a way of preventing hearing loss with aging?

We found the Mabaan people, of all ages, well-nourished, lean and erect in posture. Their diet is low in saturated fat and consists mainly of millet seed, wild dates, nuts, corn and fish. They eat almost no meat. Their mean blood cholesterol level is 160 mg/100 ml, vascular hypertension and coronary artery thrombosis are unknown and atherosclerosis is minimal. Electrocardiography revealed nothing noteworthy They seem to age more slowly than we do. Compared with many hearing studies (Glorig et al., 1957) conducted in both quiet and industrial areas, they show superior hearing with aging, particularly in the higher frequencies (Fig. 1)

In March, 1964 we performed hearing tests in Finland on people who had been under study since 1958 by Turpeinen, Karvonen and

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Fig 1 Comparison of decade udiograms (medians used) men.

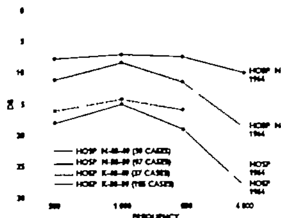


Fig 2 Air conduction means, 1964

their associates (1968) to test the hypothesis that the incidence of coronary heart disease could be decreased by a suitable change in the fat composition of the diet. The usual Finnish diet is unusually high in saturated fat, and the incidence of coronary heart disease is high. The subjects under study were patients in two large mental hospitals near Helsinki. In one the Nikkila Hospital, here called Hospital N the diet was changed in January 1959 A large part of the saturated fat was replaced by soy bean oil in skim milk, and a special type of margarine, rich in unsaturated fat, replaced the butter In the other hospital, Kellokoski, here called Hospital K, the usual Finnish diet of large quantities of whole milk and butter was maintained.

For the five years from 1959 to 1964 the diet in Hospital N (327 subjects) contained much more unsaturated fatty acids and much less saturated fatty acids than in Hospital K (254 subjects) A fall in the serum cholesterol level occurred—the level in Hospital N was on the average 51 mg/100 ml lower than in Hospital K. The adipose tissue in Hospital N contained much more linoleic acid (an unsaturated fatty acid derived exclusively from the diet) than that of Hospital K, the incidence of ECG patterns indicative of coronary heart disease was markedly and significantly lower and the coronary mortal ty also appeared to be lower although the number of deaths was too small to be statistically valid. It seemed to Turpeinen and his associates that the only significant differentiating factor in the two hospitals, contributing to the lower incidence of coronary heart disease in Hospital N was the cholesterol-lowering special diet.

In March, 1964 5 years after the diet change, we tested the hearing of these patients, without knowledge of their dietary status. They were tested in matched age groups of 40–49 and 50–59 and numbered 136 in Hospital N and 142 in Hospital K. The means for the Hospital N groups, regardless of age, are better throughout the entire audiometric range than those in Hospital K. The difference is shown in Fig. 2.

Patients aged 50–59 in Hospital N have better hearing than those 10 years younger in Hospital K. The mean in each frequency in Hospital N was significantly better than the corresponding mean in Hospital K. In each comparison the t value was well over 2.5 and thus significant to levels of 0 005 or better This study showed that the difference in hearing in the two hospitals parallels the difference in diet and the incidence of coronary heart disease.

Turpeinen stated that "An examination of the comparability of the two groups revealed differences in the two populations of reference and in some of the known risk factors, but these seemed insufficient to account for the rather

marked difference in the incidence of coronary heart disease. Hence, it was concluded that the lower incidence in Hospital N was primarily due to the cholesterol-lowering diet."

In a brilliant move to further test the validity of this conclusion and to eliminate any hidden bias, Turpeinen and his group decided to reverse the diets. In March 1965 Hospital N was returned to the normal high saturated fat diet and Hospital K was placed on the low saturated fat diet. In a personal communication Turpeinen reported that in 3½ years after this diet reversal the serum cholesterol levels in the now low saturated fat Hospital K have been between 35 mg/100 ml to 40 mg/100 ml lower than those in Hospital N The linoleic acid in the adipose tissue has changed so that at the latest sampling the mean linoleic acid content in Hospital K has risen to 32% while in Hospital N it is 13% The incidence of ECG patterns indicative of coronary heart disease has been lower during this period in Hospital K, but the figures are still too small for statistically valid conclusion.

In 1964 5 years after the beginning of the cardiological study by Turpeinen, we had found that the difference in hearing in the two hospitals ran parallel with the difference in the ...idence of coronary heart disease. In Octo... 1968, 3½ years after the diet reversal was ...tituted, we returned to further test the validity of this finding. Not all of the patients

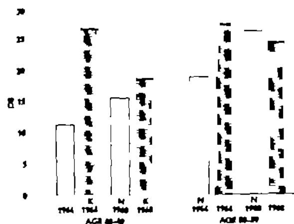


Fig 3 Air conduction means, 4 000 cps.

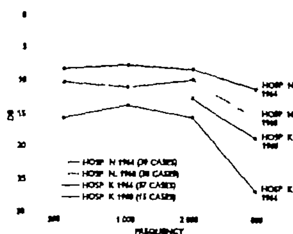


Fig 4 Air conduction means, age 40–49

tested in 1964 were available in 1968, and some of those who were available were unable to give consistent responses We were able to test, however 90 subjects in Hospital N and 75 in Hospital K whom we had tested previously We followed exactly the same procedure we had used in 1964 Reviewing that first study we found that the hearing of subjects in Hospital N then on a low saturated fat diet, had been significantly better throughout the entire audiometric range, regardless of age, than that of subjects in Hospital K which was then on a high saturated fat diet.

This 1964 difference in hearing between the two hospitals does not exist in 1968 3½ years after the diet reversal There is no significant difference, at any frequency in either age group between Hospital N and Hospital K in 1968 In 1964 the gap in hearing between the two hospitals was very significant, and suggested an association between hearing loss and the high saturated fat diet. In 1968, we find that the wide gap of 1964 has been closed, as shown in Fig. 3 There is even an over closure at age 50–59 where Hospital K has better hearing than Hospital N Thus, 3½ years after the diet reversal, the cardiological as well as the audiological findings are significantly improved in the now low-fat Hospital K.

The 1964 and 1968 statistics were compared by age group to see what kind of change had occurred in the hearing in each hospital

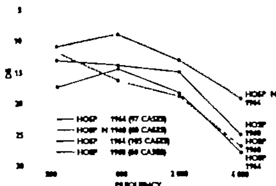


Fig. 5. Air conduction means, age 50–59.

Fig. 4 shows the age group 40–49 in 1964 and 1968. There is a trend toward a decrease in hearing in Hospital N during the 3½ year period of diet reversal and an improvement in Hospital K. These differences are visible but not statistically significant at this age group primarily because of the small number of cases. Fig. 5 shows the same means for age group 50–59. Hospital N is significantly poorer in 1968 at 1 000, 2 000 and 4 000 cps. Hospital K, however, is significantly better in 1968 at 500 and 2 000 cps, with this trend also visible at the other frequencies. In summary, the hearing of patients in Hospital N has become worse since the diet reversal, while that of patients in Hospital K has improved.

Table II. *Air conduction means* 4 000 CPS.

Age 40–49
Hospital N 1964→11.14 dB
Hospital N 1968→15.47 dB
Hospital K 1964→26.74 dB
Hospital K 1968→18.90 dB

Age 50–59
Hospital N 1964→19.22 dB
Hospital N 1968→26.68 dB
Hospital K 1964→27.82 dB
Hospital K 1968→24.97 dB

In high frequency testing at 12–24 kC the same trend appeared. *Table I shows high frequency medians for both 1964 and 1968.* Although little can be assumed from the figures at age 50–59 because of so few responses, the results at age 40–49 are interesting. There is a drop in Hospital N in both the 12 000 cps median and in the percent of response. There is a rise in the median in Hospital K and no change in the percent of response. Again we see here the important evidence of decreasing hearing in Hospital N and improving hearing in Hospital K.

Table II is a bar graph showing a comparison between Hospitals N and K at 4 000 cps, with a decrease in hearing between 1964 and 1968 in Hospital N and an improvement in Hospital K.

The mean change at 4 000 cps in both age

Table I. *High frequency medians*

	Age 40–49				*Age 50–59*			
	Hospital N		Hospital K		Hospital N		Hospital K	
kC	1964	1968	1964	1968	1964	1968	1964	1968
12	70.00 dB 85 %	84.38 dB 70 %	87.50 dB 62 %	82.50 dB 60 %	88.75 dB 59 %	MNO 25 %	MNO 42 %	MNO 17 %
14	92.50 dB 51 %	MNO 23 %	MNO 38 %	MNO 20 %	MNO 19 .	MNO 3 %	MNO 12 %	MNO 2 %
16	MNO 10 %	MNO 3 %	MNO 3 %	MNO 6 %	NR 0 %	MNO 2 %	NR 0 %	NR 0 %
18	NR 0 %	NR 0 %	NR 0 %	NR 0 %	↓	NR 0 %	↓	↓
20	↓	↓	↓	↓	↓	↓	↓	↓
No.	39	30	37	15	97	60	105	60

Table III *Mean change at 4 000 cps 1964 vs 1968*

	Hospital N		Hospital K	
Age (years)	dB	No. of cases	dB	No. of cases
40–49	+3.93	30	−7.07	14
50–59	+8.77	60	−0.37	60

\+ decrease of hearing.
− improvement of hearing.

Table V *Mean change of blood cholesterol levels 1964 vs 1968*

	Hospital N		Hospital K	
Age (years)	mg %	No. of cases	mg %	N. of cases
40–49	+53.8	19	−39.2	16
50–59	+52.4	32	−48.4	54

\+ increase − improvement.

groups at each hospital is shown in Table III. The difference between the mean change at each hospital at age 40–49 is 11 00 dB and at age 50–59 it is 9 14 dB

Furthermore, there are statistically significant differences in hearing during the 1964–1968 interval between the 40–49 age groups and the 50–59 groups in both hospitals. These differences are not due solely to the fact that the mean change for both Hospital K groups indicates improvement and that the change for both Hospital N groups indicates worsening. A highly significant statistical difference exists in each instance It is startling that the effect of aging on the hearing in Hospital K has been not only arrested—but even reversed.

In both age groups there were more subjects Hospital K whose hearing improved after diet reversal than there were in Hospital N Table IV shows the number of cases in each age group in each hospital showing decrease or improvement in hearing. Naturally a certain number of individuals will show change upon retest, and the proportion of such individuals will be about the same in each group tested. One would further expect a certain number of each group to show improvement on re measurement, even though aging had occurred in the interim. In this instance, however the number of such individuals in Hospital K is much greater than would be expected on the basis of chance variation, and the level of statistical significance is less than 0 005 well beyond the normal criteria for significance.

Examination of the figures showing blood cholesterol levels is also of great interest. In Table V we can see that the cholesterol levels of those subjects who were given hearing tests, with the mean change from 1964 to 1968, have increased in Hospital N and decreased in Hospital K. We may ask the question, within each group, what is the average change of cholesterol of those who improved compared with those who worsened? Surprisingly enough, there is not too much difference in this respect. This may encourage us to speculate along the following lines With respect to the association between serum cholesterol and coronary heart

Table IV *Change at 4 000 cps by no of cases 1964 vs 1968*

	Age 40–49		Age 50–59	
	Hospital N (cases)	Hospital N (cases)	Hospital K (cases)	Hospital K (cases)
Decrease of hearing	19	2	43	26
Improvement or no change	11	12	17	34

disease, cholesterol was originally implicated because it formed a constituent part of the atherosclerotic plaque. Later serum cholesterol was indicated on the basis of epidemiological evidence in various parts of the world. However to this day there is as yet no hard physiological evidence linking serum cholesterol as a causal agent with the development of coronary heart disease. However as we know it is an indicator of the type of diet eaten and it may be the diet, rather than the serum cholesterol which, directly or indirectly is causing the changes in hearing which we are observing in Hospitals K and N As a result, any quantitative work we do with cholesterol may yield nothing; the only important role the cholesterol would play would be to indicate that the "proper" type of diet was being consumed.

The question we asked in the beginning of this report must now be asked again. Can a reversal in diet bring about a reversal in hearing loss?

Turpeinen and his group concluded that diet is an important factor in the prevention of coronary heart disease. Likewise our audiological studies lead us to conclude that diet is an important factor in the prevention of hearing loss

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ZUSAMMENFASSUNG

Zahlreiche epidemiologische Studien auf der ganzen W lt haben gezeigt, dass bei Völkern, bei denen Coronarerkrankungen haufig vorkommen, die Tendenz zu hohen Blutcholesterinwerten besteht und die Er nährung gewöhnlich och grosse Mengen gesättigter Fette enthalt. Diese Hypothese wurde funf Jahre lang in Finnland geprüft, an zwei Versuchsgruppen im Alter von 40–59 Jahren, in zwei Irrenanstalten, wobei in der einen die übliche Kost mit dem hohen Gehalt an gesättigtem Fett weiter verabreicht wurde, während in der anderen Anstalt die Nahrung durch eine Kost ersetzt wurde, die einen wesentlich höheren Gehalt an mehrfach ungesättigten Fetten hatte.

Nach fünf Jahren war in der letztgenannten Anstalt die Häufigkeit der Coronarerkrankungen wesentlich geringer Das Gehör war in allen Frequenzen wesent lich besser Nach der fünfjährigen Versuchszeit wurde die Kost in den beiden Anstalten umgetauscht. Vier Jahre nach dem Kost Umtausch war das Gehör in der Anstalt, wo die fettarme Nahrung verabreicht wurde, gebessert, und das Gehör war schlechter in der Anstalt, wo die fettreiche Kost gegeben wurde. Die Häufigkeit der Coronarerkrankungen folgte der selben Gesetzmässigkeit. Die finnischen Forscher kamen zu dem Schluss, dass ein wichtiger Faktor in der Verhütung von Coronarerkrankungen wohl eine Kost sein mag, die arm an gesättigtem Fett ist. Unsere audiologischen Befunde zeigen gleichfalls, dass eine solche Kost Hörverlust zum Stillstand bringen kann, falls sie sogar das Gehör nicht verbessert.

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TYPE IV TYMPANOPLASTY RESULTS AFTER STAPEDECTOMY

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Abstract Of the patients on whom type IV tympanoplasty after stapedectomy has been performed since 1966 at the Otolaryngological Clinic, Ojiya Hospital, two cases are reported. Type IV tympanoplasty after stapedectomy was performed using the fascia temporalis and the pplication of a plug consisting of absorbable gelfoam dipped in a mixture of 0.5% chloromycetin and 1% hydrocortisone acetate at the oval window following the removal of the stapes facilitated the formation of a new membrane at the oval window Due to the enlargement of the small tympanic cavity cochlear fluids are set freely in motion, so that the round window membrane become freely movable following release of the displaced perilymph, resulting in a marked improvement in hearing. The formation of a prominent peak at 2 000 Hz following postoperative hearing acuity and the loading test at the oval window may indicate the minimum acoustic impedance of the inner ear Due to series of loading tests, sound energy will be con- red to be propagated chiefly through the route of ternal ear canal—oval window—inner ear It seems to the author that type IV tympanoplasty involving complete removal of the pathological stapes is worthy of notice in stapes surgery

The basic principle sought of tympanoplasty is to convey with the least possible attenuation the acoustic energy from the external ear canal to the endorgan of the cochlea through the sound conduction system of the middle ear It is well known that theoretically the improvement of hearing acuity is dependent not on the absolute magnitude of sound pressure applied on the oval window but on the relative difference in this pressure between the oval window and the round window If sound pressure on the round window is reduced to establish adequate sound protection, an improvement of hearing acuity can be attained. The type IV tympanoplasty after stapedectomy which removes the intermediation of the various ossicles is based on this principle. It is generally believed in Japan that hearing acuity after type IV tympanoplasty is not good. The detrimental factors may possibly be a considerable acoustic resistance in the region of the oval window including the pathologic stapes, and limited mobility of the round window membrane owing to hypoplastic development of the small tympanic cavity Incidentally the author encountered a patient with stapes fixation through an adhesive process and another with chronic otitis media, in the latter of whom type III tympanoplasty had to be abandoned on account of diminished distance between the promontory and the head of the stapes owing to the inclination of the stapes. Thus a type IV tympanoplasty was performed, in which, after stapedectomy the oval window region was sealed with absorbable gelfoam to accelerate a more natural formation of a tight and thin fibrous membrane and further with fascia temporalis a small tympanic cavity including the round window with the Eustachian orifice was made as wide as possible to secure satisfactory sound protection for the round window Postoperative hearing acuity was examined. Moreover by loading a small ointment applied tampon on the newly formed oval window membrane and new ear drum, change was induced in the hearing

threshold of the former and by means of these, the conduction route of sound energy was explored. The specific acoustic impedance of the inner ear as seen from the external ear canal was calculated in return from the postoperative hearing acuity

Case Report

Case 1 H. K., aged 16, school boy Left mastoidectomy at the age of 10 years. Despite of the operation, otorrhea and tinnitus occasionally developed and dysacousis gradually increased. On physical examination, there was neither otorrhea, nor tinnitus, nor dizziness, and the main complaint was dysacousis. The ear drum was cloudy showing absence of the light reflex. A calcification was visible anterio-inferiorly of the manubrium mallei. There was no perforation of the tympanic membrane but a diffuse retraction was present. The previous operative wound at the mastoid showed favourable epithelization, and the short crus of the incus was partly visible because of epithelial deficiency With the diagnosis of dysacousis due to luxation of the incudostapedial joint through an adhesive process, reoperation was carried out on July 24 1967 Hard granulation had markedly proliferated in the epitympanic cavity and around the middle ear ossicles, and the malleoincudal body showed partial carious. The incudostapedial joint was disrupted, the stapes was covered with thickened connective tissue, the head of the stapes had become partially *carious. Observation with the* operating microscope revealed no displacement of the stapes due to a non-acoustic reaction. On the suspicion of stapes fixation resulting from ringband sclerosis of the footplate of stapes, a stapedectomy was performed. After operation, a small outflow of perilymph and bleeding from the oval window was observed. Immediately the oval window region was sealed with a small piece of gelfoam, impregnated with a mixture of 0.5% chloromycetin and 1% hydrocortisone acetate, and the fascia temporalis was compressed toward the promontory with the oval window region being evaded, thus making the small tympanic cavity as wide as possible including the round window with the Eustachian orifice In this way a type IV tympanoplasty was completed, and prophylactic treatments were employed to prevent infection into the inner ear As prophylaxis against invasion of the inner ear antibiotics, peripheral vasodilators, Vitamin B_1 were administered. On the 9th postoperative day the tampon was removed, and the 10th day insufflation through the Eustachian tube was conducted daily Signs of subjective improvement of hearing acuity were gradually noted, while the formation of a small tympanic cavity became evident and dry changes in the transplantation flap also became macroscopically recognizable. No phonophobia was noted. With a 10 mg loading test on the newly formed oval window membrane, a prominent peak was observed at 2 000 Hz, and in the 50 mg loading test on the newly formed tympanic membrane, the effect of the loaded mass was slight, indicating a near normal sound protection at the round window The postoperative course is still under observation (Fig. 1)

Case 2, A. O aged 12, school girl. She had occasionally suffered otorrhea since infancy Because of her remote place of dwelling, she could not receive satisfactory treatment. At a school health examination, she was noticed to have chronic otitis media on the right side, and was brought to this clinic. The first physi*cal examination disclosed slight otorrhea, sub*total perforation of the ear drum, thickening of the remaining ear drum, fibrous thickening of the promontory membrane seen through the perforation, and inward protrusion of the osseous part of the external meatus, which narrowed the meatus. Dysacousis was ascribed to subtotal perforation of the ear drum and decreased mobility of the ossicular chain. With this diagnosis, an operation was performed on January 7 1970 under general anesthesia. The ossicular chain, which was covered with thickened fibrous tissue, could not be removed perfectly and the malleoincudal body which was

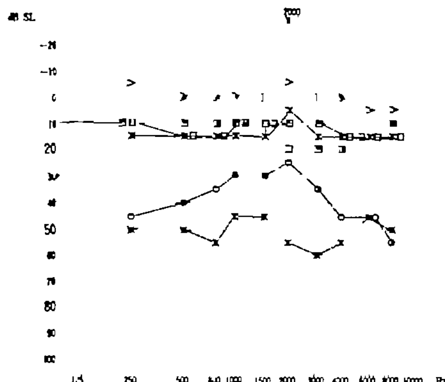


Fig. 1. H. K., aged 16, school boy. Type IV tympanoplasty involving stapedectomy for stapes fixation, resulting from ringband sclerosis of footplate of the stapes. Air and bone conduction thresholds before and after operation and after loading the newly formed oval window membrane and newly formed ear drum with 10 mg and 50 mg ointment tampons, respectively are presented. The postoperative bone conduction threshold showed improvement, showing 25 dB 2 000 Hz, and the air conduction threshold formed prominent peak of 5 dB. Also in air conduction threshold after 10 mg loading on oval window a large change was seen, a prominent peak being at 2 000 Hz. The dB SL value on audiogram is in accordance with the Japanese Industrial Standard. -- -]: Preoperative air and bone conduction thresholds; —— >: Postoperative air and bone conduction thresholds; O— —O: Air conduction thresholds after 10 mg loading on oval window: □— —□: Air conduction thresholds after 50 mg loading on new ear drum.

partly afflicted by caries, was taken out. Relatively thickened fibrous granulations had proliferated *in the region extending from the entrance* of the mastoid sinus to the tympanic cavity. The granulation was removed with care taken not to injure the facial nerve. The promontory membrane was thickened, and the stapes, being inclined, was buried in the granulation. Because of the extremely narrowed space between the head of the stapes and the promontory a type III tympanoplasty was abandoned, and only the stapes and a part of the promontory membrane near to it were removed. Immediately the oval window region was sealed with a small piece of gelfoam impregnated with a mixture of 0.5% chloromycetin and 1% hydrocortisone acetate, and the *fascia temporalis was compressed toward the* promontory and thus a type IV tympanoplasty was completed. After stapedectomy a small outflow of perilymph was observed, but no postoperative nausea, dizziness, tinnitus or phonophobia developed. On the 10th postoperative day the tampon was removed, and on the 11th day insufflation through the Eustachian tube was started. Prophylactic treatments were employed to prevent infection into the inner ear. Postoperative hearing acuity was very good. The effect of a 10 mg loading test on the newly formed oval window membrane

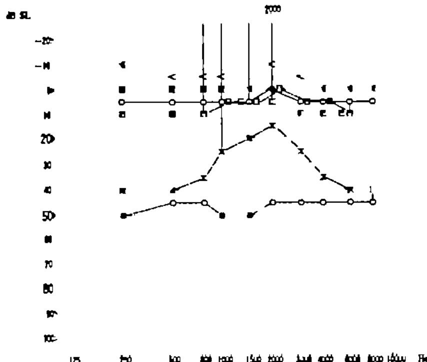


Fig. 2 A. O aged 1.., school girl. Type IV tympanoplasty Narrowed space between head of the stapes and promontory produced by inclination f the stapes in chronic otitis media, made stapedectomy imperative. Air and bone conduction thresholds before and after operation and after loading on the newly formed oval window membrane and newly formed ear drum with 10 mg and 40 mg ointment tampons, are presented. After the operation, the threshold was markedly improved, showing prominent peak of 0 dB t 2 000 Hz. Also 10 mg loading on oval window greatly lowered the air conduction thresholds, likewise forming prominent peak at 2 000 Hz. The 40 mg loading on the new ear drum effected little change. The dB SL of audiogram is in accordance with the Japanese Industrial Standard. O– – –O [Preoperative air and bone conduction thresholds· O——O < Postoperative air and bone conduction thresholds — — Air conduction thresholds after 10 mg loading on oval window· □— —□· Air conduction thresholds after 40 mg loading on new ear drum.

was remarkable, a prominent peak being found at 2 000 Hz as in case 1 The postoperative course is now under observation (Fig. 2)

DISCUSSION

Otosclerosis is said to be infrequent in Japan, generally accounting for only 0.3% of the total otopathic patients (Goto et al 1967) However if patients with stapes fixation through adhesive process, are included, the subjects for stapes surgery will be considerably greater in number

The object of type IV tympanoplasty is to establish the same sound protection for the round window as in the normal ear to produce mobilization of perilymph Now in Europe and the USA, the focus of stapes surgery seems to be found in the reconstruction of the ossicular chain after stapedectomy Concerning the use of the heteroplastic material, however Lewis (1961) Harris (1961) Smyth (1964) and Hough (1966) reported complications due to foreign body reactions. As for homoplastic material, Peer (1955) stated that autogenous bone graft was completely absorbed in several years being transformed into connective tissue But aside from the problem of complications resulting from the plastic material, there is also the problem of a complicated operative tech-

nique, which is extremely serious no matter whether heteroplastic or homoplastic material may be used. In view of this, Hough (1960) Lindsay (1961) and Kylander (1967) applied gelfoam, which was used by Correll & Wise (1945) as a hemostatic agent. According to Kylander's report, gelfoam which sealed the oval window after stapedectomy was transformed into a tight and thin fibrous membrane Under the stimulus of this report, the author applied, with strict chemotherapy a small piece of gelfoam imbued with a mixture of 0.5% chloromycetin and 1% hydrocortisone acetate to seal the oval window region after stapedectomy Further the author compressed the fascia temporalis toward the promontory evading the oval window In this way a type IV tympanoplasty was carried out Transformation of the gelfoam into a tight and thin fibrous membrane in the oval window region was considered to have taken place in about 9 or 10 postoperative days, since dizziness did not occur even transiently after insufflation through the Eustachian tube. The postoperative air-conduction threshold was markedly improved, which can be attributed not only to specific acoustic impedance of the inner ear but also the satisfactory sound protection for the round window ich was secured by the newly formed oval membrane of extremely small acoustic and by the fascia temporalis. The author did not resort to Knopfloch method as used by Wullstein, but compressed the fascia temporalis toward the promontory evading the oval window region and thus a small tympanic cavity Now it is necessary to compare the hearing acuities of the present cases with those after type III tympanoplasty had been carried out most successfully The author reported at the International Audiology Congress in Kyoto on a case of type III tympanoplasty which was considered successful since the normal movement of the stapes was elicited by the reflex of the stapedius muscle. The present results are comparable with this case both in hearing acuity after the operation and in loading tests on the oval window And the prominent peak at 2 000 Hz seems to indicate the equivalence in acoustic impedance between the stapes and the inner ear The loading was performed to elucidate the route of sound conduction after type IV tympanoplasty A small tampon with ointment was applied on the newly formed oval window membrane and on the newly formed ear drum. The effect of the mass on the oval window was remarkable, a prominent peak being formed at 2 000 Hz while the air conduction threshold was lowered. On the new ear drum, however scarcely any change in the air conduction threshold was observed. It was consequently assumed that after type IV tympanoplasty sound energy would be conveyed chiefly through the oval window and that the mobilization of the perilymph and hearing acuity would be dependent on the magnitude of acoustic resistance at the oval window region. For the audiometer (Rion, AA 33) which was acceptable by the Japanese Industrial Standard, and which was used in this clinic, the relation between dB SL and dB SPL at 2 000 Hz is expressed as follows:

$$\text{dB SPL} = \text{dB} + 17\ \text{dB}$$

If the plane wave is assumed to fall vertically on the newly formed oval window membrane, sound conduction loss (dB SPL) is expressed by the formula.

$$\text{dB SPL} + 10 \log_{10} \frac{0.031 \times Zc}{4 \times Zo}$$

Zc denotes specific acoustic impedance of the inner ear as seen from the newly formed oval window membrane, and specific acoustic impedance Zo of the air is taken as 42 acoustic ohms, and the area of the newly formed oval window membrane as 3.2 mm^2 By this formula, Zc against 2 000 Hz is calculated as follows.

$$Zc\ (5\ \text{dB SL},\ 2\,000\ \text{Hz}) = 84 \times 10^4\ \text{acoustic ohms}$$

It is thus known that at 2 000 Hz the best mobilization of perilymph and the best transmission of the surface wave of the perilymph

is established This is of interest in connection with findings reported last year by the author (1969) that on the application of a strong acoustic stimulus to human ear the largest displacement of the stapes was produced at 2 000 Hz through the reflex of the stapedius muscle.

In the present work, the number of cases was small, and the period of follow-up observation was short, and the author considers it nec essary to pursue the work further But it seems to the author that type IV tympanoplasty involving complete removal of the pathological stapes is worthy of notice in stapes surgery

ZUSAMMENFASSUNG

Es wird über zwei von mehreren Fällen von Tympanoplastik Typ IV im Anschluss an Stapedektomie berichtet, die seit 1966 in der Hals-Nasen-Ohren-Klinik des Ojiya Krankenhauses behandelt wurden. Bei der Operation wurde die Temporalis-Faszie ver wendet, und ein aus Gelatineschwamm bestehender Pfropfen am ovalen Fenster der in einer Mischung von 0.5% Chloromycetin und 1% Hydrocortisonl Acetat getränkt wurde, förderte im Anschluss an die Steigbügelentfernung die Bildung einer neuen Membran am ovalen Fenster Aufgrund der Vergrösserung der kleinen Paukenhöhle werden in der Schnecke Flüssigkeiten in Bewegung versetzt, wodurch die Membran des runden Fensters nach dem Freisetzen der verdrängten Labyrinthflüssigkeit frei beweglich wird. Dies wiederum führt zu einer deutlichen Ver besserung der Hörschärfe. Das Ausprägung eines prominenten Gipfels bei 2 000 Hz nach postoperativen Hörschärfe- und Belastungstests scheint die minimale akustische Impedanz des Innenohres anzudeuten. Auf grund einer Reihe von Belastungstests wird Entstehung der Schallenergie hauptsächlich auf dem Weg: äusserer Gehörgang, ovales Fenster und Innenohr angenommen. Der Verfasser ist der Ansicht, dass Tympanoplastik Typ IV mit vollständiger Entfernung des pathologischen Steigbügels bei Steigbügeloperationen berücksichtigt zu werden verdient.

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ALTERATIONS OF ROUND WINDOW RECORDED N_1 BY SELECTIVE SECTIONS OF THE COCHLEAR AND VESTIBULAR NERVES

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Abstract Electrical potentials recorded from the round window membrane of the cat cochlea are divided into neural (N and N_2) and non-neural, hair-cell components (cochlear microphonics) N and N are grossly altered by the sectioning of the entire VIII cranial nerve. Selective sectioning of the cochlear and vestibular portions of the nerve at the internal meatus enabled a cause and effect relationship to be established. N–N cleft distortion, N amplitude increase and blockade of cochlear efferent (o.c.b.) stimulation was produced by sectioning of the vestibular fibers and cochlear efferents. Sectioning of the afferent fibers produced only a minor cleft distortion. Since initiation of the lesion does not depress N and stimulation of either o.c.b. does, it is presumed that the changes produced are not related to discharge of the o.c. bundles but rather to removal of tonic activity

A convenient means of measuring the cochlear response to sound stimuli is to record the potentials from the round window According to Davis (1957), this recording represents the hair cell (CM) and neural (N_1 and N_2) activity of the specific area recorded near the electrode.

Fisch & Ruben (1962) found that N_1–N_2 cleft distortion occurred when the entire VIII nerve was sectioned at the internal auditory meatus. Daigneault et al (1968) confirmed their findings and observed that the N_1–N_2 cleft distortion also occurred when VIII nerve conduction was blocked by application of local anesthetics.

The purpose of this report is to present the results of selectively placed lesions of the nerves at the internal auditory meatus upon the round window recorded cochlear responses to click stimuli. The role of the efferents in the production of the N_1 and N_2 responses is the primary concern in these investigations.

MATERIAL AND METHODS

The experimental animal used was the cat. The stimulation and recording procedures used were those previously described (Daigneault & Brown, 1969) The animals were anesthetized with an intraperitoneal injection of Dial Urethane (65 ml/kg, Ciba Pharmaceutical) and placed in an Industrial Acoustics Co Model 400A sound chamber after exposure of the left internal auditory meatus Body temperature was maintained through the intermittent use of a heating pad.

Sectioning of the various portions of the nerve trunks (Fig. 1) was performed without interruption of the recording of cochlear responses to clicks. Because the light source used to illuminate the visual field during section produced a 60 cycle interference, the recor[illegible] were distorted for 5–10 sec. Surgical

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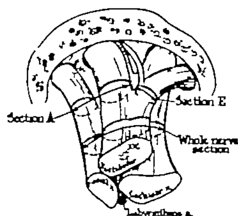


Fig. 1. Structures at the internal auditory meatus. The placement of various lesions in the area of the internal meatus are indicated. Section *E* contains the fibers of the olivocochlear bundle as they form Oorts Bundle. Section *A* includes some of the afferent fibers of the cochlear nerve as well as the facial and vestibular nerves. Notice that some bone was removed in the drawing to allow demonstration of the course of the fibers as they penetrate the bony foramena.

of the internal auditory meatus did not produce any abnormality in the tracing. The blood supply to the cochlea was not interrupted during the surgery since the tracing continued unchanged for more than 1 hour subsequent to the sections. In 7 animals (Series A) the efferent section was made before the afferent section. In Series B (5 animals) the procedure was reversed. The method of sectioning was based upon the description of Spoendlin (1966 *a*).

Olivocochlear bundle (o.c.b.) stimulation was performed in 3 cats in each of the two series of experiments. These animals were immobilized with gallamine triethiodide (Flaxedil, Davis & Geck) as previously described by Brown et al. (1969). When o.c.b. stimulation was performed, hollow ear bars were used to enable the click stimuli to be presented to the cochlea. Acoustic and o.c.b. stimulation parameters were established using the method of Brown et al. (1969). The control tracing of such an experiment is presented in Table I.

Measurements were made on the round window recorded responses as described in Fig. 2. Paired, one-tailed *t* tests were performed on the data using $P < 0.05$ as the acceptable level for indication of a significant difference.

RESULTS

The changes produced by the various lesions are presented in Table I. When the cochlear efferents and vestibular nerve were sectioned first (Section *E*, Series A) the cleft voltages and N amplitudes were significantly increased and o.c.b. stimulation was rendered ineffective. Subsequent section of the cochlear afferents (Section *A*, Series A), after 2–3 hours recovery, produced only a relatively small alteration of the new control tracing. The cleft voltage was significantly elevated, but not to the extent produced initially, and no other significant change was noted.

When the sequence of sections was reversed (Series B), the results of the cleft changes were also reversed. The first procedure (Section *A*) altered the cleft voltage, but did not alter the N_1 height voltage or the effectiveness of the o.c.b. stimulation. When the second lesion (Section *E*) was made after the recovery period, o.c.b. stimulation was no longer effective, and the cleft voltage was greatly altered. Under these circumstances, the increase in N_1 amplitudes occurred but was not statistically significant.

Depression of N was never observed immediately after section, indicating that injury produced by the sections did not cause discharge of the cochlear efferents.

DISCUSSION

Disruption of conduction in all the nerve fibers at the internal auditory meatus produces a characteristic distortion in the N and N potentials as recorded from the round window. This phenomenon reverts towards an approximately normal state during a two hour period (Daigneault et al., 1968). If the blood supply to the cochlea is interrupted as reported by Perlman et al. (1959), the evoked neural potentials are rapidly lost. During these experiments,

Table I *Modification of cochlear round window recording produced by selective sectioning of the cochlear and vestibular nerves*

Series A consisted of 7 animals in which the cochlear efferents were sectioned (Section E) followed by afferent section (Section A). In Series B, the sections were made in reverse order on 5 animals. The time allowed for recovery between sections was 2–3 hours. The parameters of measurement were: Cleft change in μV, *N* height in μV, Delay in msec, and o.c.b. in dB attenuation of the *N* response to a click produced by efferent stimulation (reference 1.0 V = 0.0 dB). The values are expressed with ±S.E.M. The set of cochlear responses presented with Series A are photographs of responses from one cat taken before and immediately (within 30 sec) after the sections. The same is true for Series B.

Series A

	Control	Section E	P value	Recovery Control	Section A	P val
Cleft	194 ± 50	81 ± 33	<0.05	118 ± 34	1 ± 19	<0.05
N Height	357 ± 82	434 ± 106	<0.05	324 ± 86	301 ± 96	
Delay	1.04 ± 0.08	1.04 ± 0.09		1.05 ± 0.06	1.03 ± 0.07	
o.c.b.	20.5 ± 2.1	2.2 ± 0.93	<0.05	3.4 ± 1.9	2.3 ± 1.3	

Series B

	Control	Section A	P value	Recovery Control	Section E	P value
Cleft	142 ± 32	24 ± 24	<0.05	42 ± 25	125 ± 22	<0.05
N Height	263 ± 46	270 ± 43		268 ± 44	304 ± 50	
Delay	1.00 ± 0.03	1.03 ± 0.02		1.02 ± 0.03	1.06 ± 0.03	
o.c.b.	21.3 ± 0.9	22.0 ± 0.2		22.7 ± 1.2	−0.8 ± 0.7	<0.05

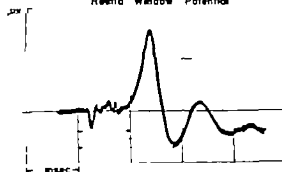


Fig. 2

The measurements reported were made according to the sites indicated. (1) The delay from the beginning of the MC to the beginning of N. (2) *N* height, the amplitude of N above zero, and (3) the cleft, the measure of the height of the tracing at the time indicated after the beginning of MC. Note—Negative refers to below 0, not polarity of recording.

the amplitude of the N_1 potential remained the same or increased, indicating that the cochlear blood supply was not interrupted. The consistency of the delay time of *N* throughout all experiments is also indicative of a sound physical condition of the cochlea.

Production of stepwise lesions of the efferent and afferent nerves at the internal meatus enables further evaluation of what portion of the VIII nerve is responsible for the changes produced by whole nerve section. At the internal meatus the efferent fibers are found to be in the vestibular branch and form Oorts Bundle, which joins the cochlear nerve (Fig. 1) in their more distal course. When the fibers associated with Section *E* are severed, the tracing is affected in a way similar to that produced by whole nerve section or local anesthetic blockade

When the o.c.b is stimulated subsequent to the efferent section, it is not functional as a depressant of N_1. Some abberrant efferent fibers may be present in the structures severed by Lesion *A* as distortion is produced by that lesion (Table I). However the alteration of the tracing is most pronounced after Lesion *E* and appears to be due to the removal of the efferent component. It is possible that the round window electrode is recording a vestibular response to the click that is superimposed on N_1 and N_2. This seems unlikely for the following reasons. (1) N_1 and *N* are thought to represent cochlear afferent activity only (Davis, 1957) (2) a vestibular event of sufficient magnitude to be recorded from the perilymph at the round window would be several times larger at its point of origin than the naturally occurring cochlear afferent activity because of the distances involved in the volume conductor and (3) this vestibular event would have to be phase-locked at the same time after the acoustic stimulus that *N* and *N* appear. Therefore, it is proposed that the cochlear efferents are the source of generating the alteration of the cleft of the round window tracing.

The N_1 and *N* components of the round window recorded response to a click are thought to represent the sum total of cochlear afferent activity at the basal turn of the cochlea. Alteration of the cleft between *N* and N_2 after disruption of the cochlear efferents might be due to removal of a tonic efferent influence, causing a change in the temporal pattern of discharge of individual auditory afferents to the click. Fex (1962, 1965) demonstrated that tonic or resting activity is found in approximately 10% of the contralateral and 40% of the homolateral o.c.b. fibers of the anesthetized, curarized cat. The observation that the *N* amplitudes increase when the efferents are sectioned also suggests that tonic efferent activity exists. The finding that *N* increases after strychnine (1) indicates tonic efferent activity as well. Rossi et al (1964) found a greater red c tion of microphonics when the homolateral o.c.b. was cut than when the contralateral o c b was cut, confirming Fex' finding of a higher level of tonic activity in the homolateral than in the contralateral o.c.b. Thus it seems that tonic efferent activity does exist in anesthetized animals. However although strychnine produces an increase in *N* it does not affect the shape of *N* and N_2. Therefore, either strychnine is incapable of blocking the efferent sites involved in production of the cleft alteration or disruption of tonic efferent activity per se, is not involved in generation of the cleft alteration.

Spoendlin (1966 *b*) suggested that ephaptic transmission may occur between afferent and efferent dendrites since their unmyelinated parts lie in very close proximity. Perhaps removal of tonic efferent activity increases the possibility of this phenomenon occurring. Cleft alteration might then occur due to this ephaptic interaction producing either a change in the firing pattern of the afferent dendrites or a post-synaptic potential that is related to a different pattern of efferent activation after removal of the tonic efferent activity. Desmedt (1965) reported the results of stimulating the efferent nerve in a deaf albino cat. He recorded a round window tracing which contained a quick component of nerve potential properties following o c.b stimulation. It was described as being a "strychnine sensitive potential dependent upon synaptic interactions between two neural components of the inner ear and could represent post-synaptic inhibitory potentials elicited by activated olivocochlear terminals in the membranes of auditory dendrites. The recording presented by Desmedt is remarkably similar to the pattern obtained when the *N* and *N* potentials obtained after section of the efferents are subtracted from the control *N* and *N* potentials (Fig. 3). It is possible that increased ephaptic interaction between afferent and efferent dendrites could lead to a post-synaptic potential in the afferents being superimposed on the normal *N* and *N* potentials, resulting in the cleft alteration observed in these experiments. However the possibility that altered ephaptic transmission after disrup-

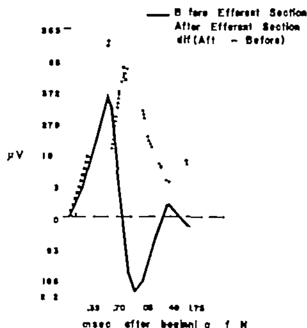


Fig 3 Alteration of *N* and *N* by efferent section
The before and after curves are the graphic presentations of measurements made before and immediately after efferent section on one of the cats in Series A. The measurements were made every 0 018 msec and at every point at which a peak occurred. Note—Negative refers to below 0, not polarity of recording.

tion of the efferents produces a change in the pattern of firing of the afferents cannot be ruled out at the present time

From our previous investigations we have that, when local anesthetics are used n the trunk of the VIII nerve, the altered tracing persists for the duration of action of these agents (Daigneault et al 1968) In contrast, after section the round window tracing returns toward a normal appearance within 2 hours. What causes this recovery is not known.

It is reasonable to expect that the recording from the round window represents the condition of the afferent dendrites and the impinging efferents. The status of the recording is definitely modified by such conditions as the depth of anesthesia, type of anesthetic and degree of efferent activity What the efferents seem to be able to affect during "normal" operation is the cleft as well as the N_1 amplitude. How this is associated with the effects of o.c.b stimulation upon the height of N_1 is unknown. Further questions are therefore raised concerning the role of the cochlear efferents.

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ZUSAMMENFASSUNG

Elektrische Potentiale die von der Membran des fenestra rotunda der Katzen Cochlea abgeleitet werden, können in neurale (N und N_1) und nicht neurale Haarzellen Komponenten (cochleare Mikrophonien) eingeteilt werden. N und N werden nach Durchschneidung des gesamten achten Hirnnervens stark verändert. Selektive Unterbrechung des cochlearen und vestibularen Anteiles des Nervs am meatus acusticus internus ermöglichte die Aufstellung eines Ursache und Wirkungs-Verhältnisses. Veränderungen der N_1–N Spalte, Erhöhung der V Amplitude und Blockade des Effekts der Reizung der cochlearen efferenten Fasern wurden durch die Unterbrechung der vestibularen Fasern und der efferenten cochlearen Fasern erzeugt. Durchschneidung der afferenten Fasern verursachte no geringfügige Veränderungen der Spalte. Da die Unterbrechung des Nerven nicht zu einer sofortigen Erniedrigung von N führt während dieser Effekt durch Reizung des olivo-cochlearen Bündels hervorgerufen werden kann, darf daraus gefolgert werden, dass die beschriebenen Veranderungen nicht mit den Entladungen von den olivo-cochlearen Bündeln zusammenhängen, sondern auf der U terbrechung tonischer Einflüsse der efferenten Fasern beruhen.

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CRITICAL EVALUATION OF THE ACTION OF THE POSTERIOR CRICO-ARYTENOID MUSCLE, UTILIZING DIRECT EMG-STUDY

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Abstract. In the present investigation the electrical activity of the posterior crico-arytenoid muscle has been studied by direct electromyography In the course of direct laryngoscopy under neurolept analgesia 10 patients, 4 males and 6 females, have been examined for the evaluation of unilateral vocal cord immobility The normally mobile side was utilized for the study of the action of the posterior crico-arytenoid muscle. This muscle showed a basic resting electrical activity as well as a rhythmic respiratory fluctuation, with an inspiratory rise of electrical activity on deep respiration. During closure of the glottis, as in phonation and sphincteric actions, the posterior crico-arytenoid muscle exhibited a considerable degree of electrical activity It is suggested that the posterior crico-arytenoid muscle is not solely an abductor muscle, and though it has an abducting during forcible inspiration it does not seem the sole abductor Several structural properties motor control of the small internal laryngeal muscles are discussed. No evidence can be found as a specific arrangement for the two antagonistic muscle systems, abductors and adductors.

The action of the posterior crico-arytenoid muscle as the sole dilatatory muscle of the glottis has been the subject of universal agreement for several decades By virtue of the work of its different groups of fibres this muscle is supposed to glide the arytenoid cartilage outwards over the cricoid and to rotate its vocal process out around the vertical axis of the crico-arytenoid joint, thus widening the glottis. This concept of function is based on mechanical analysis of the muscle s pull on the crico-arytenoid joint. Many of the presumptions concerning the mechanics of this joint have already been declared to be fallacious (Frable 1961 von Leden & Moore, 1961 Ardran & Kemp 1966) Moreover the study of the action of each laryngeal muscle in isolation, as if behaving singly seems to be an oversimplification of the problem A complex, delicate balance of the laryngeal structure is maintained by the simultaneous activity of several internal and external muscles at all instances The role played by the latter group has long been neglected, although a few publications have focused light on a possible and even significant influence of these muscles on the size of the glottis and vocal fold tension (Fink et al., 1956 Zenker 1964)

In addition to the evident uncertainty about the action of the posterior crico-arytenoid muscle laryngeal muscles in general have presented *a subject for controversy as regards the details* of their action. Since the advent of electromyography as a tool of exploring muscular action many workers have applied this test to laryngeal muscles (Wedell et al., 1944 Feinstein, 1945–46 Portmann et al., 1955 Fink et al 1956 Faaborg-Andersen & Buchthal, 1956 Faaborg-Andersen, 1957 Greiner et al., 1958 Sawashima et al., 1958 Baldan, 1963 Kotby 1967 Hiroto et al., 1968)

The results obtained by various investigators are by no means consistent. That the basic resting activity of the posterior crico-arytenoid muscle, as well as members of the adductor group of muscles, increases during inspiration

and even more during forced inspiration has been reported by Faaborg-Andersen & Buchthal (1956) and Faaborg-Andersen (1957). These investigators found that only the posterior crico-arytenoid muscle shows also increased activity during forced expiration. This paradoxical behaviour of the posterior crico-arytenoid muscle was, however not recorded by other investigators. Felnstein (1945–46) described a sort of "tone" in the abductor muscle, even when the cords were adducted. On phonation Faaborg-Andersen (1957) and Hiroto et al. (1967) described an absent or very slight electrical activity in the posterior crico-arytenoid muscle.

In an attempt to evaluate these findings, percutaneous electromyographic recordings have been obtained from various laryngeal muscles, including the posterior crico-arytenoid (Kotby 1967). The results obtained from the investigations of the latter showed that this muscle plays a relatively passive role in widening the glottis. In addition this muscle showed significant activity during phonation and sphincteric activity of the larynx.

These results are quite contradictory to current text-book teaching. This, together with the awkward anatomical position of the posterior crico-arytenoid muscle which makes percutaneous approach exceptionally difficult and somewhat uncertain, necessitates a confirmatory direct electromyographic study of this muscle.

MATERIAL AND METHOD

The conditions required for the present experiments entail adequate sedation of the patient, allowing the passage of a Magill laryngoscope with the least possible discomfort and irritation. The patient's consciousness should not be reduced to a degree that interferes with his response when asked to perform certain tasks. These requirements are fulfilled in neurolept analgesia. A premedication in the form of Droperidole 0.10–0.15 mg/kg intravenously is given 1 hour before examination. On the arrival of the patient in the theatre pharyngo-laryngeal topical analgesia is administered, using Lidocain spray. Immediately before proceeding with the test, the patient is given Phentanyl 0.05–0.1 mg intravenously.

The present series comprises 10 patients, 4 males and 6 females. Their ages range from 22 to 71 years. The subjects were examined in the course of direct laryngoscopy for the evaluation of unilateral vocal cord immobility. The normally mobile side was utilized for the study of the action of the posterior crico-arytenoid muscle.

The electrical activity of the muscles was recorded by a DISA electromyograph. Both the three-channel (13A84) and the two-channel (14C02) apparatus was used. The amplifiers of the two apparatuses have almost the same input specifications. In this way the results recorded by either of them could be taken safely for comparative purposes.

After exploring the larynx, the post-cricoid region was visualized. The tip of the laryngoscope was passed well beyond the arytenoid masses until the lamina of the cricoid with its covering structures could be seen and felt. At this level the whole extent of the posterior crico-arytenoid muscle was clearly seen. Concentric needle electrodes (DISA 13K51) were passed down through the laryngoscope, utilizing crocodile forceps. They were inserted through the mucosa on the back of the cricoid lamina on either side of the middle line to penetrate 3–4 mm into the posterior crico-arytenoid muscle. The forceps and the laryngoscope could then be withdrawn, thus leaving the patient least disturbed. The chances of electrode displacement were much less than expected. Apart from vigorous swallowing movements and coughing laryngeal actions could be tested with little risk of electrode displacement.

The patient was left to regain the fully relaxed state. The resting activity of the muscle was recorded. The patient was then asked to breathe deeply. The respiratory fluctuations thus recorded were checked against the phases of the respiratory cycle in different ways. An

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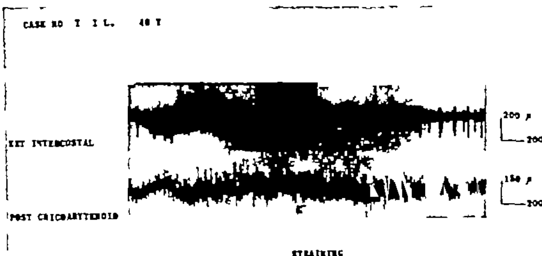


Fig 5 During straining there is forced expiratory effort, which entails a marked reduction of activity in the "inspiratory" external intercostal muscle, as shown in the upper trace. Simultaneously the "inspiratory" posterior crico-arytenoid muscle shows considerable rise of electrical activity

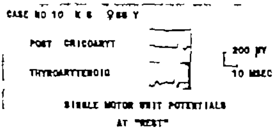


Fig 6 Individual motor units recorded from the posterior crico-arytenoid muscle (*upper trace*) and the thyro-arytenoid muscle (*lower trace*), demonstrating that the electrodes picking up activity from different motor units in these two muscles.

be explained as an effort on the part of the small internal laryngeal muscles attached to the crico-arytenoid joint to increase the stability of the joint in face of an external driving force probably from the strap muscles of the neck and the so-called *functional chain* (Fink et al., 1956 Zenker 1964)

During emission of sound the vocal cords are tightly adducted By purposive exhalation efforts the subglottic pressure is increased until it can push apart the adducted vocal cords, along variable portions of their length with the resultant expulsive puff of air The same applies to the sequence of events during sphincteric actions of the larynx. During the pharyngeal stage of swallowing and during coughing and straining, the larynx closes tightly either for protective purposes or for building up subglottic pressure The recording of considerable electrical activity from the posterior crico-arytenoid muscle during phonation and sphincteric actions is thus considered an important challenge to the conventional view that this muscle is a pure dilatatory muscle of the glottis and that in this respect it is antagonistic to the adductor muscles

Accordingly it can be concluded that the posterior crico-arytenoid muscle is not solely an abductor muscle, and though it may have an abductive effect during forcible inspiration it does not seem to be the sole abductor In this connection it is also reasonable to assume that the function of the posterior crico-arytenoid muscle and other small internal laryngeal muscles is primarily postural. They may be considered as braces to stabilize the crico-arytenoid joint, without necessarily moving it.

Some structural properties of the small internal laryngeal muscles are in support of the assumption of non-specificity of action of these

muscles, as regards adduction and abduction of the vocal folds. Unlike the extra-ocular muscles the medullary neurons controlling the small internal laryngeal muscles are hitherto not precisely mapped for the separate muscles. An accepted segmental arrangement, however suggests that the caudal parts of the nucleus ambiguus control all the small muscles attached to the arytenoid cartilage regulating movements of adduction and abduction (recurrent laryngeal nerve, sixth branchial nerve) More cranially the centre controlling the crico-thyroid muscle is found (external laryngeal nerve, fourth branchial nerve) (Luchsinger & Arnold, 1965) Moreover the internal topography of the recurrent nerve does not admit of the isolation of a specific abductor bundle The bundles are rather well intermingled (Sunderland & Swaney 1952) *The recurrent nerves enter the* larynx behind the crico-arytenoid joint. They may enter the larynx unbranched or they may have as many as six branches (Bowden, 1955) In most cases, the nerve divides into two main branches—an anterior and a posterior The latter was thought to supply the abductor fibres (Cracovaner 1954) There is, however considerable controversy and a great many individual variations (Keros & Nemanic, 1967) [illegible] extra laryngeal division of the recurrent [illegible] is into motor and sensory branches. It [illegible] not into abductor and adductor branches" (Williams, 1954) No general agreement can be reached about a fixed pattern which serves a special bundle from any special branch to the posterior crico-arytenoid muscle

Unlike ordinary skeletal muscles, the motor units of the small internal laryngeal muscles consist of a small number of muscle fibres. Such a strikingly low innervation ratio fits the type of muscular action required from the laryngeal muscles. These muscles are known to be non-weight-bearers and to perform delicate actions entailing rapid contractions. The small internal laryngeal muscles have been found to possess more than one irregularly arranged motor end-plate The highest number of multi motor end-plate fibres exists in the vocalis muscle (thyro-arytenoid) The posterior crico-arytenoid muscle possesses the lowest number of these multi motor end-plates, while the crico-thyroid and lateral crico-arytenoid muscles occupy an intermediate position (Rossi & Cortesina, 1965)

The morphological arrangement of the muscle fibres in different internal laryngeal muscles presents various patterns Some fibres run directly between the points of attachment, others end on intermediate tendons or on perimysium of other muscle fibres. This arrangement holds true for all members of the internal laryngeal muscles (Zenker 1964) although Rossi & Cortesina (1965) *emphasize* that there is a difference between the posterior crico-arytenoid muscle and the adductor group They consider the latter to consist only *of fibres running from one insertion to the other* This is, however contrary to the findings of Zenker (1964) in the vocalis muscle

A certain gradation in physical properties of the internal laryngeal muscles has been described. The vocalis muscle is the fastest contracting muscle, the posterior crico-arytenoid has the slowest and most sustained contraction, while the crico-thyroid occupies an intermediate position (Hast, 1966, 1967 *a* 1967 *b*) This reflects an equivalent variation of the type of metabolism of these muscles. The slow sustained contraction of the posterior crico-arytenoid muscle demands a high aerobic metabolism. This muscle has been found to have a rich capillary blood supply and a high density of mitochondria The fast muscle on the other hand has both aerobic and anaerobic types of metabolism (Mira & Vidi 1966) These differences, however indicate a different degree of activity of the individual members of the internal laryngeal muscles concerning control of vocal fold tension, rather than a structural segregation of these muscles into a unique abductor muscle and a group of adductors.

From the preceding analysis of the motor control of the internal muscles of the larynx no evidence can be found as to a specific arrangement for the two antagonistic muscle systems.

It is also evident that the small internal laryngeal muscles, essentially alike, are unique in structure, which enables them to perform a tonic slow contraction as well as rapid phasic changes

Neurohistological studies led by several authors (Piquet & Barets, 1960 Lucas Keene, 1961 Bianconi & Molinari, 1962) have been able to demonstrate positively the presence of a spiral type of proprioceptor in the small internal laryngeal muscles in man as well as animals. Neurophysiological investigations point also to the presence of two different types of mechanoreceptors in the larynx, low threshold rapidly adapting articular mechanoreceptors of phasic excitation, and the receptors responsible for the myotatic reflexes (Abo-El Enein & Wyke, 1966) These have two different functions, facilitatory and inhibitory which may arise from different types of receptors. In general these muscle receptors are of low threshold and very slowly adapting, thus capable of maintaining tonic activity

The proprioceptive outflow of the larynx is strictly unilateral and is carried mainly by the superior laryngeal nerve (Eyzaguirre et al., 1966) In spite of the fact that the recurrent laryngeal nerve carries some of this afferent outflow yet it seems that in the small internal laryngeal muscles, contrary to other body muscles, the motor and afferent functions are grossly served by two different nerves.

The emergence of the assumption of nonspecificity of function of the small internal laryngeal muscles necessitates a reasonable substitute for the mechanics of laryngeal respiratory dilatation In other words: What can be the abducting force of the vocal folds Moreover to what extent can the presented assumption serve to evaluate observations made in cases of mobility disturbances of the vocal folds, and in what way can it help to explain *Semon* s law?

The answer to these questions demands extensive supplementary neurophysiological investigations, some of which are being attempted at present.

ZUSAMMENFASSUNG

In dieser Untersuchung ist die elektrische Aktivitet des M. crico-arytenoideus post. bei direkter Elektromyographie studiert worden. Im Verlauf von direkter Laryngoskopie unter neuroleptischer Analgesie wur den 10 Patienten, 4 Manner und 6 Frauen, für die Beurteilung von einseitiger Stimmbandlahmung unter sucht. Es wurde die normal bewegliche Seite für das Studium von der Aktion des M. crico-arytenoideus post. benutzt. Dieser Muskel zeigte sowohl eine basale elektrische Ruheaktivitet als auch rhythmische respiratorische Schwingungen, mit einer inspiratorischen Steigerung der elektrischen Aktivitet unter tiefer Respiration. Während der Glottisschliessung, wie unter Phonation und Sphinkteraktivitet, zeigte der M. crico-arytenoideus post. einen bedeutenden Grad von elektrischer Aktivitet. Es wird angedeutet, dass der M crico-arytenoideus post. nicht allein ein Abduktormuskel ist, und obwohl er einen Abduktions- ffekt unter kraftiger Inspiration hat, scheint er nicht der alleinige Abduktor zu sein. Verschiedene struk turelle Eigenschaften der motorischen Kontrolle der kleinen inneren Kehlkopfmuskeln werden diskutiert. Es sind keine Anhaltspunkte für eine spezifische Anordnung der beiden antagonistischen Muskelsysteme, Abduktoren und Adduktoren, gefunden worden.

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ELECTROMYOGRAPHY OF THE ESOPHAGUS AND ITS CLINICAL APPLICATIONS

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Abstract The authors have succeeded in simultaneously recording electromyograms of the upper mid- and lower esophagus by new intraluminal lead method designed for the examination of esophageal motility in human subjects. The following action potentials ere observed in normal cases: (1) spike-burst appeared occasionally in the mid- or lower esophagus at rest; (2) action potentials followed deglutition i.e. spike-burst propagating from the upper to lower esophagus and repetitive spike-bursts from the lower esophagus, (3) spike-burst elicited by instillation of drug solution, (4) spike-burst licited by esophageal wall distension by air insufflation. The lectromyographic findings of patients with esophageal disease were clarified in each case. In case of idiopathic esophageal dilatation characteristic spike-burst was found in the lower esophagus which seemed to indicate the presence of cardiospasm, and many sporadic spike discharges were also observed in the mid- and lower esophagus after mecholyl injection.

In physiological study of the esophagus as well as in the clinical diagnosis of esophageal disease, electromyographical examination offers different clinical benefits than those derived from examination of the esophagus by esophagoscopy radiology or recordings of intraluminal pressures because the motility of the esophagus is detectable at a muscular level. However to date there has been no method available for electromyography of the esophagus in human subjects. The reason is the difficulty in consistently obtaining an accurate electromyogram without iatrogenically inducing a lesion in the esophagus.

Electromyographic studies of the esophagus in animals were reported in detail by Brücke & Inouye (1912) Brücke & Satake (1913), Yokosawa (1956) Tada (1959) Inouye (1966) Sato et al. (1967) and many other workers. However only Nomoto (1964) have reported on the clinical applications of electromyography to study the esophagus. In his report an outer polyethylene tube was first inserted into the esophagus, and another inner tube, which contained two conducting wires equipped with a silver wire 0 1 mm in diameter at one end, was introduced inside the outer one. Electromyography of the lower esophagus was carried out through the conducting wire. He demonstrated electromyograms of the lower esophagus in healthy individuals as well as in patients with idiopathic esophageal dilatation, but his electromyography of the esophagus were considered to be inadequate for clinical use because it was not simultaneously recording electromyograms of the upper mid- and lower esophagus and its technique was complicated.

This report describes electromyography of the esophagus in human subjects using intraluminal leads capable of simultaneously obtaining electromyograms of the upper mid- and lower esophagus without inducing lesions in the esophagus. In addition electromyographical patterns of esophageal motility in normal cases and electromyographical findings of patients with esophageal diseases are reported.

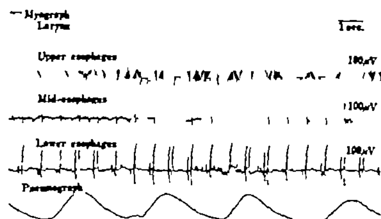


Fig 1 Electromyogram of the esophagus in the resting state.

METHOD

Electrode

An electrode tube was prepared utilizing a rubber tube (6 mm in outer diameter 4 mm in inner diameter and 55 cm long) coiled with a silver wire 300 μ in diameter at 1 cm intervals. Three pairs of bipolar ring electrodes were used to simultaneously record the motility of the upper mid and lower esophagus. A long as well as a short electrode tube was made In the long tube the position of the electrode was at 18 19 29 30 and 43 44 cm from the standard point. In the short tube the electrode was located at a distance of 15 16, 25 26, and 38 cm from the standard point. Each elec c leads to an amplifier through an enamlled copper wire inside the tube A vinyl tube was introduced inside an electrode tube until its end reached a position between the upper and middle electrodes where the vinyl tube was run out through a hole to the surface of the electrode tube Then, a sample solution or air was infused into the esophagus through this hole during examination. A thread was passed through the electrode tube in such a way that it ran out 5 cm outside the tube at the position of the middle as well as the lower electrode Therefore when the thread was pulled up the electrode tube curved in the part where the middle and the lower electrodes were located, so that the electrodes were fixed to adhere closely to the esophageal wall.

Procedure

The electrode tube was introduced with the subject in a sitting position and passed far enough so that the standard point of the tube was level with the incisors and the electrodes were then fixed by pulling back on the thread. Then, electromyography was carried out with the subject supine after the position of each electrode was confirmed by radiographic examination. The act of deglutition as well as respiration was recorded by a five channel polygraph and, at the same time, electromyography of the upper mid and lower esophagus was carried out. The act of swallowing was signalled by recording the action potential of the accessory muscles of deglutition through cutaneous electrodes. Respiration was followed by recording chest movements by use of strain gauge transducer All electromyogram recordings were performed at a paper speed of 1 cm or 2.5 cm using 0 003 sec as a time constant

OBSERVATIONS

Electromyograms of the Esophagus in Normal Case

Resting state (Fig. 1)

A persistent spike discharge with an amplitude of about 100 μV was recorded in a lead from the upper esophagus, possible indicating activity of the cricopharyngeus muscle. Records

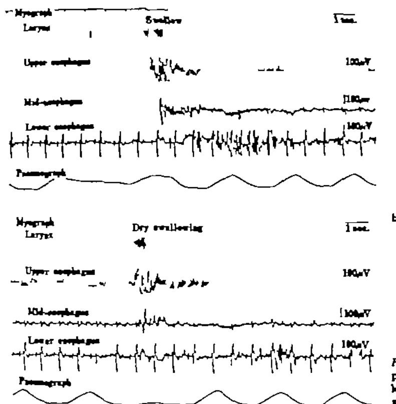


Fig. 2. Electromyogram of the esophagus during water and dry swallowing. (*a*) Water swallowing. (*b*) Dry swallowing.

from leads of the mid- and lower esophagus were found to be influenced by some electrocardiographic factors, and seldom showed the sporadic spike-burst which indicated the activity of the esophagus. These spike-bursts were considered to be localized reflex action potentials due to presence of the electrode tube, although a spontaneous action potential by esophageal automatism can not be ruled out.

On swallowing

(1) On swallowing 5 ml of water (Fig. 2 *a*)

When a subject swallowed 5 ml of water a spike-burst successively appeared in the larynx, upper mid- and lower esophagus at succeeding time intervals. In the electromyogram of the upper esophagus, an irregular fluctuation of the base line of the record with laryngeal movements, which was presumed to be an artifact due to the cricopharyngeal muscles relaxing, and then slight increase of intensity of the persistent spike discharges was noticed. The spike-burst from the mid-esophagus was classified into two types. the first spike-burst appeared immediately after laryngeal movement, the second appeared approximately 2 sec after the first one disappeared. The spike-burst from the lower esophagus occurred approximately 2 sec after that of the laryngeal muscle. These action potentials, which the authors termed a propagative spike-burst, may indicate Meltzer's primary peristalsis of swallowing. In the lower esophagus, the propagative spike-burst was followed by a repetition of spike bursts which seemed to indicate a localized peristalsis. This kind of action potential was termed repetitive localized spike-bursts of the lower esophagus on swallowing. The spike bursts from electromyograms of the upper mid- and lower esophagus on swallowing were found to be different in their properties from each other

The spike-burst from the upper esophagus consists of spike discharges of a low amplitude (about 200 μV) and high frequency (about 20 spikes per second). The spike-burst from the

mid-esophagus, the first spike-burst consists of spike discharges of slow frequency and the second one consists of spike discharges of a low amplitude (about 50 μV) at a high frequency In comparing the spike-burst by the upper esophagus with that of the lower area, it was observed that the spike-burst of the lower esophagus consists of spike discharges of a higher amplitude (about 500 μV) and lower frequency (about 10 spikes per sec) These differences in the properties of spike discharges arise from the fact that the upper and lower esophagus consist of striated muscle and smooth muscle respectively and that the mid-esophagus is located in a transitional area between striated muscle and smooth muscle

(2) On dry swallowing (Fig. 2 *b*)

A propagative spike-burst was observed upon dry swallowing as shown in Fig 2 *b* A spike burst in the lower esophagus appeared approximately 5 sec after that in the laryngeal muscle. This time interval of 5 sec is much longer than that observed when swallowing water No repetitive spike-burst was found in an electromyogram of the lower esophagus on dry swallowing. The propagative spike-burst of the lower esophagus did not consistently appear during dry swallowing. It is well known that geal peristalsis on swallowing is centrally ed The remarkable difference in the agative time for spike-bursts between dry and water swallowing may be due to the presence of a localized reflex action potential when a subject swallows water

Electromyogram of the esophagus when a solution or air was instilled into the esophagus

(1) When water or a drug solution was instilled into the esophagus (Fig. 3)

Fig. 3 *a* shows an electromyogram of the esophagus obtained when 5 ml of water was instilled into the esophagus for 5 sec through a vinyl tube A spike-burst was found in the lower esophagus when water was instilled into it, but then there were some cases where the intensity of the spike-burst was not constant

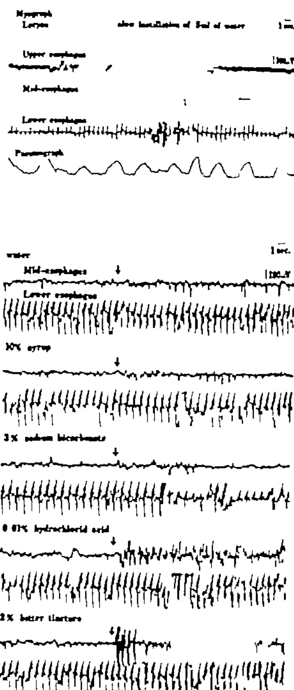


Fig 3 Electromyogram of the esophagus with instillation of water or drug solution into the esophagus. ( ) Water instillation. (*b*) Drug instillation.

and an action potential failed to appear Fig. 3 *b* shows the appearance of spike-bursts when water 10% syrup 3% sodium bicarbonate 0 01 . hydrochloric acid or 2 bitter tincture

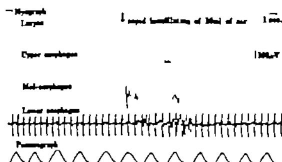


Fig. 4. Electromyogram of the esophagus with rapid air insufflation into the esophagus.

in 5 ml volumes were instilled into the esophagus for 5 sec, indicating the intense spike bursts with hydrochloric acid and bitter tincture. The intensity of spike-bursts was found to differ according to the properties of the drug solution. Therefore, the spike-burst is considered to be a localized reflex action potential induced by stimulation of the mucous membrane of the esophagus, but could not be an action potential due to stimulation by the distension of the esophageal wall.

(2) When air was rapidly insufflated into the esophagus (Fig. 4)

Fig. 4 shows an electromyogram of the esophagus obtained when 10 ml of air was insufflated as rapidly as 0.5 sec through a vinyl tube with a syringe. A rapid air insufflation resulted in the appearance of a spike-burst in the lower esophagus. This type of spike-burst was, in many cases, observed approximately 5 sec after the air insufflation as shown in Fig. 4 and was considered to be a localized reflex action potential due to local distension and hence stimulation of the esophageal wall.

Effects of autonomic drugs

An electromyogram of the esophagus was compared before and after the injection of 0.1% adrenaline (0.013 ml/kg), 1% pilocarpine (0.013 ml/g), 0.1% atropine (0.013 ml/g), mecholyl (1 mg/60 g) or benzylimidazolin (20 mg). When pilocarpine was injected, a repetitive localized spike-burst from the lower esophagus upon swallowing was intensified and a localized reflex spike-burst, which appeared when a solution or air was infused, was also found to be intensified. These spike-bursts were reduced to a lesser extent when atropine was injected. The effects of the other drugs on the electromyogram of the esophagus were not clear.

Electromyograms of the Esophagus in Clinical Cases

A case of extirpation of the cervical esophagus

A 58-year-old man with cancer of the cervical esophagus had the pharynx and cervical esophagus extirpated. Electromyography of the lower esophagus was performed by the insertion of an electrode tube through the cervical esophageal fistula. A spike burst recorded from a lower esophageal lead was observed about 5 sec after pharyngeal motility started following dry swallowing (Fig. 5). It was found that an action potential from the lower esophagus appeared although the cervical esophagus was removed. This may support the fact that esophageal peristalsis upon swallowing is not propagated via the muscular fiber of the esophageal wall, but propagates via extrinsic nerves.

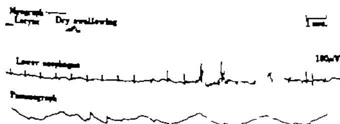


Fig. 5. Electromyogram of the lower esophagus in patient with extirpation of the cervical esophagus. Record on dry swallowing. Act of swallowing showing spike-burst in the lower esophagus.

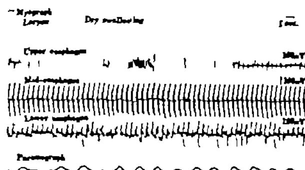


Fig 6 Electromyogram of the esophagus in a patient with cicatricial stenosis in the mid-esophagus. Record on dry swallowing. The spike-burst in the upper esophagus is excessive. A propagative spike-burst could not be observed in the mid- and lower esophagus.

A case of unilateral vagal paralysis

A 54-year-old woman whose chief complaint was a disorder of swallowing visited our department. This case was diagnosed as a jugular foramen syndrome, because of paralysis of the left IX, X and XI cranial nerves. Radiological examination revealed that barium was retained in the hypopharynx and then was aspirated into the bronchus because of a disorder in the second phase of swallowing. The electromyogram showed an irregular pharyngeal motility which continued as long as 16 sec. This indicates that the second phase of swallowing was not proceeding smoothly. However, on one occasion water reached the esophagus, a propagative spike-burst by the esophagus appeared in a normal pattern (Fig. 6). A spike-burst by the lower esophagus occurred 2.8 sec after that by the upper esophagus. The authors concluded from these results that unilateral vagus nerve paralysis will not interfere with a propagative spike-burst by the esophagus, possible because the esophagus is controlled by bilateral vagal innervation as shown in a report by Inouye (1966).

A case of cicatricial stenosis

A 37 year-old man drank a cup of an acidic cleanser solution for tiles by mistake. Three months later, he found it difficult to eat solid food, and 4 months later it was difficult for him to drink water. A retrograde dilatation along a string was performed through a gastric fistula. During treatment, electromyography of the esophagus was carried out in such a manner that the electrode tube was introduced into the esophagus through the gastric fistula and retrogressively pulled up by a thread which passed from the fistula to the mouth, because it was difficult to insert the electrode tube from the mouth owing to a high degree of stenosis. The electromyogram of the upper esophagus during swallowing showed a marked spike-burst with an amplitude of 200 μV which continued for approximately 6 sec, and which was found not to propagate to the mid- and lower esophagus (Fig. 7). After the stenosis was improved by retrograde dilatation, an electromyogram upon swallowing indicated that the persistent spike-burst by the upper esophagus had disappeared and that a propagative spike burst by the mid- as well as the lower esophagus had appeared. The propagative spike-burst by the mid- and lower esophagus failed to appear during swal-

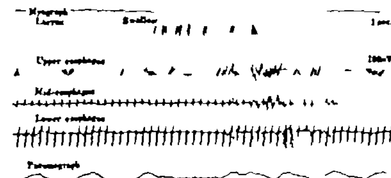


Fig 7 Electromyogram of the esophagus in a patient with left vagal nerve paralysis. Record on water swallowing. The second phase of deglutition was disturbed but the propagative spike-burst from the upper to the lower esophagus occurs normally.

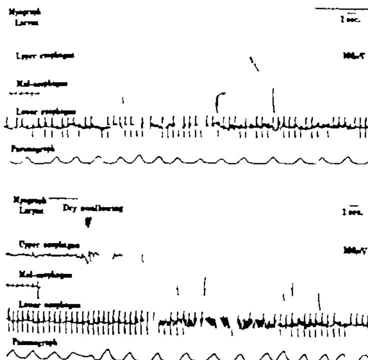


Fig. 8. Electromyogram of the esophagus in patient with idiopathic esophageal dilatation. This is re cord when the patient complained of intense substernal pain following esophagoscopy. (*a*) A characteristic spike-burst reminiscent of cardiospasm appeared in the lower esophagus. (*b*) On swallowing, the spike-burst t the abdominal esophagus was intensified.

lowing in the presence of the stenosis. This can be attributed to the reflex suppression of the spike-burst by the mid and lower esophagus caused by a centripetal impulse directed to the medulla oblongata owing to the presence of a persistent strong contraction in the upper esophagus.

A case with intrathoracic esophago-gastric anastomosis

A 47-year-old woman drank 3 ml of a sodium hydroxide solution by mistake Three months later, she found it difficult to swallow liquid. Radiography of the esophagus revealed that barium did not pass the middle constriction of the esophagus. She underwent intrathoracic esophago-gastric anastomosis. The esophagus was removed through the middle constriction, and the stomach was pulled up into the thorax. Electromyography of the esophagus was per formed 1 month after operation. The position of the inserted electrodes were confirmed by radiography As a result, the upper electrode was found to be located in the upper esophageal part in which slight dilatation was noted, and the middle electrode was about 5 cm down the sutural part inside the thoracic stomach When she drank 3 ml of water a spike-burst in the upper esophagus was observed, and subsequently spike-bursts appeared from the middle electrode. It is difficult to conceive that the peristaltic movement was being propagated to the transposed stomach. Therefore, the spike burst by the transposed stomach was considered to be a localized reflex action potential caused by stimulation of the mucous membrane

A case of idiopathic esophageal dilatation

A 22-year-old man had substernal burning as well as vomiting these past 3 years when eating. This case was diagnosed as idiopathic esophageal dilatation. Since electromyographic ex amination of the abdominal esophagus was nec essary it was confirmed by radiography be fore each examination that the lower electrode was located in the abdominal esophageal region. When he swallowed water or it was infused, it was retained in the esophagus. Then, each recording was carried out after the re tained water was repeatedly removed with an

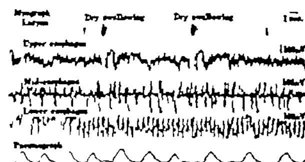


Fig 9 Electromyogram of the esophagus after injection of mecholyl in the patient in Fig. 8 Sporadic spike discharges appear in the mid- and lower esophagus.

aspirator through the vinyl tube in place for water infusion. An electromyogram of the esophagus upon swallowing 3 ml of water showed the appearance of propagated spike-bursts in lesser intensity than those in normal cases. The appearance of a repetitive localized action potential in the lower esophagus was not clearly observed. The presence of a propagative spike-burst indicates that the failure in the passage of esophageal contents was not due to the absence of esophageal peristaltic movements.

When he complained of a pain in the lower sternal area after he underwent esophagoscopy an electromyogram of the abdominal esophagus obtained at rest showed the characteristic spike-rsts of a low amplitude and high frequency shown in Fig. 8 *a*. On swallowing, these spike bursts in the abdominal esophagus were intensified (Fig. 8 *b*) and then disappeared in about 15 sec coincident with the resolution of the pain in the sternal region. This was considered to b an unusual action potential induced by the resultant abdominal esophageal dilatation by esophagoscopy Further it seems to be an electromyogram of cardiospasm

In this case it was confirmed by radiography that the peristaltic movement was found to be remarkably activated by the injection of mecholyl as reported by Akakura et al. (1964) An electromyogram after metholyl injection showed frequent sporadic spike discharges from the mid as well as the lower esophagus (Fig. 9) A localized reflex action potential decreased in this case when a drug solution or air was infused.

DISCUSSION

On intraluminal leads

The authors have employed an intraluminal lead method for electromyography of the esophagus in normals as well as clinical cases. An advantage of this method is that an electrode can be placed at an objective region without inducing any lesion in the esophagus. However it can happen that an electrode will artificially be dislocated owing to deglutition and esophageal peristalsis. Therefore, the authors have paid particular attention to fix the electrodes in an objective position. when a thread passed through the electrode tube was pulled, the tube curved in a part where the electrodes were located, so that they were fixed to adhere closely to the esophageal wall As a result, the action potentials in the electromyogram obtained by the intraluminal lead method were found to be characteristic of the upper mid-, and lower esophagus Thus, the authors concluded that an accurate electromyogram of the esophagus could be obtained by our intraluminal electromyography

Basic patterns of electromyogram of esophagus in normal cases

Esophageal motility was observed by electromyography The following four types of action potentials were obtained.

(1) A sporadic spike-burst was found from the electromyogram of the mid as well as the lower esophagus observed in a subject at rest. This spike-burst may indicate a localized reflex action potential due to the insertion of the electrode tube into the esophagus, although a spontaneous action potential by esophageal automatism can not be ruled out.

(2) A propagative spike-burst upon swallowing successively appeared in the upper mid- and lower esophagus at succeeding time intervals. On water swallowing, a propagative spike-burst in the lower esophagus was found to be accompanied by the repetitive appearance of

several spike-bursts, but no spike-burst in the lower esophagus appeared on repetition with dry swallowing.

(3) When a solution was infused into the esophagus, a localized reflex spike-burst due to stimulation of the esophageal membrane was observed.

(4) When air was rapidly infused into the esophagus, a localized reflex spike-burst appeared through stimulation by esophageal wall dilatation.

Propagation of esophageal peristalsis upon swallowing

In the propagative spike-burst which appeared in the esophagus following water swallowing, the spike-burst by the upper esophagus consists of spike discharges of a low amplitude at a high frequency. The spike-burst by the lower esophagus consists of spike discharges of a high amplitude at a low frequency. This can be attributed to the fact that the upper and lower esophagus differ by striated muscle and smooth muscle respectively. As to spike bursts in the *mid*-esophagus, the first spike-burst consists of spike discharges of a low frequency and the second one which appeared approximately 2 sec after the first spike-burst disappeared consists of spike discharges of a low amplitude at a high frequency. This may indicate that the mid-esophagus is located in a transitional zone between striated muscle and smooth muscle. By the character of the spike discharge, the first spike-burst is of smooth muscle origin and the second one of striated muscle origin.

As shown in Fig. 2 *a* the spike-burst in the lower esophagus as well as the first spike-burst in the mid-esophagus occurred before the second spike-burst appeared in recording leads from the mid-esophagus. For there seem to be the two types of esophageal peristalsis in water swallowing; one is a peristaltic movement which propagates from the upper to the mid-esophagus, and the other is a peristaltic one which starts in the mid-esophagus and propagates to the lower esophagus before the former reaches the mid-esophagus.

During dry swallowing a spike-burst consisting of spike discharges of low amplitude and high frequency was observed in an electromyogram from the mid-esophagus, and subsequently a spike-burst appeared in the lower esophagus (Fig. 2 *b*). It is most likely that a peristaltic movements in the upper, mid- and lower esophagus propagates in succession.

From observations on peristaltic movements in dry and water swallowing, esophageal peristalsis following deglutition can be classified into two types: (1) a peristaltic movement successively occurring in the upper, mid- and lower esophagus, (2) the peristalsis which starts in the mid-esophagus following water swallowing which propagates to the lower esophagus before the peristalsis starting in the upper esophagus propagates to the mid-esophagus.

ZUSAMMENFASSUNG

Es gelang den Verfassern gleichzeitig Elektromyogramme des oberen, mittleren und unteren Speiseröhrenabschnitts mit Hilfe eines neuen intraluminalen Speiseröhren-Elektromyographen aufzunehmen, der zur Untersuchung der Beweglichkeit der menschlichen Speiseröhre entworfen wurde.

A. In normalen Fällen wurden die folgenden Entladungen beobachtet:

(1) Ein Aktionspotential erschien gelegentlich im Ruhezustand im mittleren und unteren Speiseröhrenabschnitt.

(2) Aktionspotentiale gefolgt von Verschlucken, d.h. ein vom oberen zum unteren Speiseröhrenabschnitt sich fortpflanzendes Aktionspotential und wiederholte Aktionspotentiale im unteren Speiseröhrenabschnitt.

(3) Aktionspotential hervorgerufen durch Einflössen einer Medikamentlösung.

(4) Aktionspotential ausgelöst durch Wandausdehnung der Speiseröhre als Folge von Lufteinblasen.

B. Die elektromyographischen Befunde der Patienten mit Speiseröhrenerkrankungen wurden in jedem einzelnen Falle geklärt. Bei einer idiopathischen Speiseröhrenerweiterung wurde in dem unteren Speiseröhrenabschnitt ein charakteristisches Aktionspotential festgestellt, das auf Kardiospasmus zu deuten schien, und nach Einspritzen von Mecholyl wurden in dem mittleren und unteren Speiseröhrenabschnitt ebenfalls viele sporadische Aktionspotentiale beobachtet.

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GRANULAR CELL MYOBLASTOMA OF THE LARYNX

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Abstract. Five cases f granular cell myoblastoma of the larynx have been reported. One occurred in a boy of 12 years; another in female of 36 years, recurred after 14 years. The histology has been described and in 3 cases the interstitial angular body" cells of Bangle were demonstrated. The histochemical findings were considered to be in general accord with those of previously reported investigations. Theories of aetiology and pathogenesis have been briefly reviewed. Sixty-four cases of granular cell myoblastoma of the larynx were found in the literature, no details being available in four of these. The total number of recorded cases (69) represents more than 10% of myoblastomas in all sites. The peak age incidence of the laryngeal cases was in the fourth decade with male predominance of nearly 2 1

The first person to describe granular cell myoblastoma as a distinct histological entity was Abrikossoff (1926) though Murphy et al. (1949) drew attention to six earlier accounts. Over 500 cases of this so-called tumour have now been recorded and, of these, approximately 35% have occurred in the tongue, 30% in the skin whilst the remainder have been found in almost every other site in the body The lesion, characterized by large granule-filled cells, may occur in any age group but those encountered in children have involved predominantly the skin, as noted by Kessler (1963) and Apted (1968) and there has been a marked tendency for multiple involvement in contrast with the picture seen in later life According to Colberg & Hubay (1963) multiple lesions occur in about 4% of cases, most frequently but by no means exclusively cutaneous in origin. The existence of malignant variants is much disputed and estimates vary widely but it seems likely that the total number of cases exhibiting malignancy is probably less than 20 (Hunter & Dewar 1960 Caby et al 196? Colberg & Hubay 1963 Mackenzie, 1967) Familial incidence has been recorded by Strutzler (1963) Baraf & Bender (1964) and Eberle & Conley (1968) Some American records suggest that the lesion may have an affinity with negroes (Fust & Custer 1949 Vance & Hudson, 1969) Whilst the most common single site for granular cell myoblastoma is the tongue, a number of cases of laryngeal origin have been reported and the purpose of the present paper is to record a further 5 cases involving this anatomical site and to review those already published.

MATERIAL AND METHODS

The 5 cases were seen and treated in the Royal National Throat, Nose and Ear Hospital between 1948 and 1970. Cases 1 and 4 were included but not reported in detail by Epstein et al (1957) in an account of laryngeal polyps. Material for histological examination was confined to formalin-fixed, paraffin-embedded tissue. In addition to routine staining procedures, histochemical studies were performed as far as availability of tissue permitted. The investigations included tests for carbohydrate, lipoid and protein material.

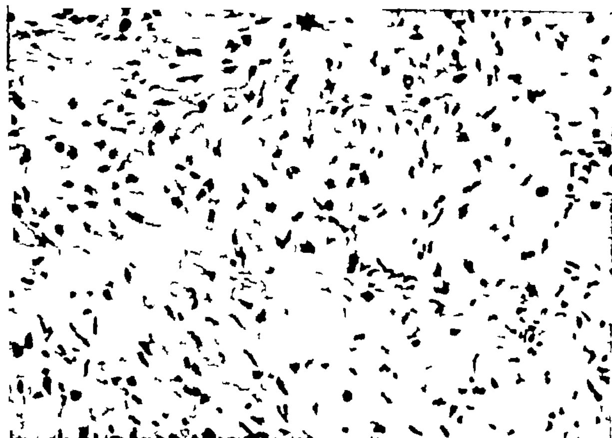


Fig 1 Case 1 Granular cell myoblastoma of the larynx. Granular cells forming occasional nests with scanty blood vessels and interstitial fibrous tissue. Haematoxylin and Eosin, × 270.

Case Reports

Case 1

A male aged 32 years was seen in August 1948 when he complained of a retching sensation in the throat of recent onset. Examination showed a small rounded swelling over the left vocal process. Removal was carried out under general anaesthesia. The patient was never seen again after his discharge from hospital and all trace has been lost

Histology showed densely packed, poorly defined cells with abundant cytoplasm and slightly shrunken nuclei. The cells, which were filled with palely eosinophilic granules, were often straplike or spindle-shaped and occasionally arranged in poorly formed whorls or nests. The nuclei showed some pleomorphism, sometimes being hyperchromatic and irregular whilst occasional nucleoli were to be seen. There were no identifiable nerve fibers and the overlying epithelium showed no abnormality (Fig. 1)

Case 2

A boy aged 12 years was first seen in July 1957 with a history of hoarseness of 6 months duration. Direct laryngoscopy was carried out under general anaesthesia and a small polyp was removed from below the anterior commissure The patient was seen at regular intervals and minor webbing in the anterior commissure was noted on each occasion but vocal cord movement was always full and equal He was last seen in December 1965 at the age of 20 years, prior to emigration when there was no evidence of recurrence

Histology revealed a more or less continuous



Fig 2 Case 4 Granular cell myoblastoma of the larynx. Group of large granular cells embedded in fibrous tissue. Movat pentachrome stain, 145

sheet of large, moderately eosinophilic cells with abundant granular cytoplasm and relatively small darkly staining nuclei. The cells tended to form columns and nests but the individual boundaries were often poorly defined. Interspersed blood vessels and strands of connective tissue occupied clefts between the columns and groups of cells. No nerve fibres could be identified. Many of the cells showed a more or less homogeneous granulation but in some aggregates almost as large as the nuclei could b- seen and occasional vacuoles were present.

Case 3

A male aged 62 years was first seen in January 1968 with a history of pain in the left side of the throat for 2 months He complained also of huskiness and pain in the left ear On examination, a nodular mass was found arising from the left pharyngo-epiglottic fold and removal was carried out under general anaesthesia. The patient was last seen in January 1970 when there was no evidence of recurrence.

Histology revealed a tightly packed mass of moderately eosinophilic cells with abundant, granular cytoplasm and well stained, medium sized nuclei. The cells, which were often poorly delineated and covered by pseudo-carcinomatous epithelium, appeared to vary in size and shape and were interspersed with fine strands of collagen. The nuclei were mainly round or oval but sometimes hyperchromatic and irregular and nucleoli were often present. The intracellular granules were predominantly of the same size but occasional larger aggregates were seen.

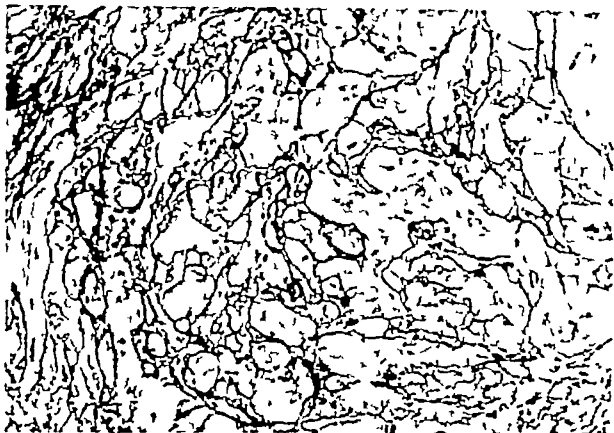


Fig 3 Case 3 Granular cell myoblastoma of the larynx. Reticulin stain showing pericellular fibrils. 280.

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A female aged 36 years was first seen in October 1956 when she presented with intermittent hoarseness of 6 months duration. A polypoid swelling was removed from the posterior half of the right vocal cord, using direct laryngoscopy under general anaesthesia. In February 1959 a smooth swelling was still present on the right vocal cord which was, however completely mobile and a biopsy at that time revealed no evidence of neoplasia. In February 1960 a further biopsy was also negative In July 1969 the patient, now aged 49 years, complained of some discomfort on swallowing but her voice was normal. Examination showed a nodular swelling to be present on the right vocal cord and a white ulcerated tumour measuring about 8 mm in its largest diameter was removed using micro-laryngoscopy under general anaesthesia.

Histology of the material removed in 1956 showed a markedly hyperplastic, pseudocarcinomatous epithelium beneath which were closely packed pale staining cells with abundant granular cytoplasm and relatively small nuclei. The epithelial changes were so marked that an initial diagnosis of carcinoma was made but later amended. The intracellular granules were somewhat loosely arranged, mainly homogeneous in size but with occasional larger aggregates

The material removed in 1969 showed a markedly fibrotic mucosa containing scattered granular cells many of which were found sandwiched between coarse strands of collagen (Fig. 2) from which, however they appeared to be sharply delineated The nuclei were round

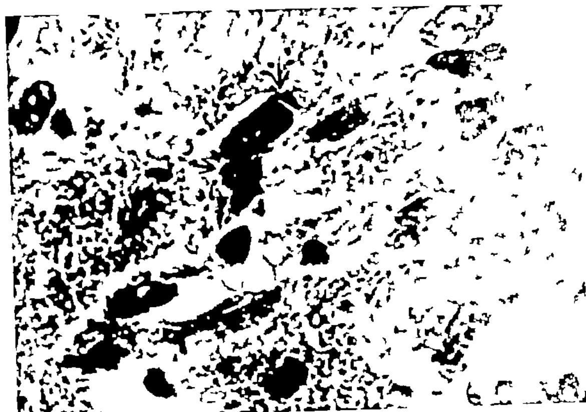


Fig 4 Case 3 Granular cell myoblastoma of the larynx. Rod-shaped angular bodies in non-granular cell. PAS stain, 1 600.

or oval, with or without nucleoli, well stained and often multiple The intracellular granules appeared mainly uniform in size with occasional larger aggregates.

Case 5

A male aged 31 years was first seen in February 1970 when he presented with hoarseness of 2 months duration. An adequate view of his larynx was only obtained by direct laryngoscopy under general anaesthesia when the left vocal cord was found to be covered by a baggy mucosa which was stripped from the middle two thirds and sent for histology The patient continues under observation.

Histology revealed large straplike cells which appeared to form branchir columns widely separated by loosely arranged fine strands of connective tissue The lls were moderately eosinophilic and packed with granules amongst which larger aggregates could be seen. The nuclei were round, oval or angular in outline whilst a few were pyknotic and irregular The overlying epithelium showed pseudocarcinomatous changes. No nerve fibres could be identified.

Histochemistry

The further study of histological and cytological detail was dependent on more specific staining methods The fine connective tissue framework was more obvious using Masson's trichrome technique than with Van Gieson stain. Reticulin staining revealed a network of fibrils surrounding groups of cells and also extending between individuals (Fig. 3) Histochemical investigations were limited by the lack of frozen sections and the fact that only hae

matoxylin- and eosin-stained sections were available in Case 1 and the original biopsy in Case 4 In the remainder the intracellular granules were studied in greater detail With Periodic acid-Schiff staining, the small granules varied in their reaction from negative to weakly positive whilst the larger aggregates stained more strongly positive and the picture was not altered appreciably by prior treatment with diastase. In Case 5 the PAS reaction was inhibited by prior acetylation. Alcian Blue produced a faint greenish colouration of the cell contents When this dye was combined in the pentachrome stain devised by Movat (1955) a similar staining gave a useful contrast with adjacent deeply stained connective tissue (Fig. 2) Apart from occasional scattered granules from disrupted mast cells Toluidine staining showed no evidence of metachromasia Staining with Sudan Black was negative in Cases 2, 3 and 4 but faintly positive in Case 5 which also showed a similar reaction with Oil Red. Millon reagent gave a negative reaction with Case 3 but was positive in Case 5

In addition to the aggregate granules, other strongly positive PAS-stained material was seen in Cases 2, 3 and 5 but not in Case 4 The material was in the form of rods, round polygonal bodies usually occupying smaller which did not appear to be otherwise granular (Fig. 4) and might be termed interstitial cells

DISCUSSION

In the context of Ear Nose and Throat practice it is worth noting that the granular cell myoblastoma has been recorded as occurring in the pharynx (3 cases) larynx (64 cases) trachea (4 cases) bronchus (33 cases) and oesophagus (4 cases) but none from any of these sites has exhibited malignant behaviour In one case reported by Rojer (1965) there were multiple bronchial and subcutaneous lesions and thickened nodules were present on both vocal cords although no histology was carried out on the latter

Sixty cases of laryngeal involvement have been located in the literature and the relevant details are summarized in Table I Four other cases (Fust & Custer 1949 Hollinger & Johnston, 1951 Bangle 1952 Vance & Hudson, 1969) have been omitted from the table owing to insufficient information but, together with those of the present series, they bring the total number of cases with laryngeal lesions to nearly seventy giving a relative incidence of laryngeal "myoblastoma" of over 10% In this anatomical site the condition appears to be a disease of earlier rather than later life the peak age incidence being in the fourth decade with a male predominance of nearly 2 1 Five cases have occurred in children of 16 years and under one of which (Cracovaner & Opler 1967) was the only case with involvement of the anterior commissure The lesion of Case ? in the present series was also in this region. The posterior third of the vocal cord was affected in 24 cases whilst 3 were subglottic in origin 6 were supraglottic and a further 8 cases had lesions arising from the arytenoid itself The size of the tumour has varied greatly and, as might be expected, hoarseness has been the predominant symptom.

The treatment of granular cell myoblastoma is local removal but in cases of laryngeal involvement special problems arise Two cases, both in children have required laryngectomy (MacNaughton & Fraser 1954 Ottosson, 1964) Eight other cases have required laryngofissure (Somers & Farinacci, 1953 Balshi, 1960 Chiappe 1961 Lyons et al., 1962 Pope, 1965 André et al., 1966 Schneider et al., 1969) thus making a total of 10 cases in which major surgical procedures were performed for a benign lesion All but one of these cases had involvement of the posterior commissure or the ventricle and part of the thyroid ala also had to be removed on two occasions in order to facilitate adequate resection. Three cases, in which the tumour was freely mobile presented as emergencies with laryngeal stridor Radiotherapy has been given to both cutaneous and laryngeal lesions with-

out benefit. Baraf & Bender (1964) treated two patients suffering from cutaneous lesions with injections of Triamcinalone and claimed some temporary improvement.

The histological diagnosis of granular cell myoblastoma is liable to be obscured by the curious propensity of the lesion to induce irregular proliferation of the overlying epithelium. Although this change is now widely accepted as "pseudo-carcinomatous" the high index of suspicion attached to vocal cord biopsies may persuade an observer to make a diagnosis of carcinoma and overlook or dismiss the granular cells merely as histiocytes. Clearly it should be axiomatic that carcinoma is never diagnosed in the presence of this granular cell lesion. The eosinophilic property of the granules is very variable and may be rather feeble so that when the cells lie amongst collagen fibres they may be overlooked. Alkek et al. (1968) found Movat pentachrome stain to be useful in this context and, in Case 4 of the present series, the full extent of the granular cell involvement was not appreciated until this stain was used (Fig. 2)

The granular cell myoblastoma remains a highly controversial lesion in which the issues have not been clarified by the welter of observations which has been recorded since the condition was first established as an entity. At the centre of the controversy is the lack of agreement on the cell of origin, the derivation of the granular cell having been variously ascribed to

1 Skeletal muscle cells (Abrikossoff 1926 1931 Willis, 1967)
2. Histiocytes (Leroux & Delarue, 1939 Azzopardi, 1956 Whitten, 1968)
3 Fibroblasts (Pearse, 1950)
4 Nerve sheath cells (Fust & Custer 1949 Bangle, 1952 Fisher & Wechsler 1962 Alkek et al. 1968)
5 Mesenchymal cells (Moscovic & Azar 1967 Toto & Restarski, 1967 Aparicio & Lumsden, 1969)

Inconstant association with muscle and apparent segregation of the two types of cell under the electron microscope have rendered origin from muscle a less favourable theory but similar arguments have been reversed in more recent times to support a neurogenic origin. Hardly less unanimous are the views on the nature of the lesion. Recognition of malignant granular cell tumours is clearly relevant to the supposed neoplastic origin of the benign lesions. Mackenzie (1967) reported one and reviewed 12 other acceptable malignant tumours. Acceptance of malignant variants would be a point in favour of the neoplastic nature of the much more numerous benign lesions. On the other hand, their rejection as wrongly diagnosed sarcomas (Azzopardi, 1956) opens the way to consider the benign condition as a metabolic disturbance (Shear 1960 Sobel & Churg, 1964) a thesaurismosis (Azzopardi, 1956) or as has been suggested by many simply a degenerative phenomenon

A mass of data has already accumulated from the study of the intracellular granules by various workers. Histochemical studies have been reported by Pearse (1950), Bangle (1952) Azzopardi (1956), Fisher & Wechsler (1962) Halsken & Langer (1962) Sobel & Churg (1964), Alkek et al. (1968) and Aparicio & Lumsden (1969). There is general agreement that the intracellular granules are PAS-positive in varying degree, the more intense staining being seen in the larger aggregates whilst previous treatment with diastase has little effect, suggesting the absence of glycogen. Sobel & Churg (1964) emphasized the weak staining of the smaller granules and contrasted this with the more strongly positive reaction of intracellular granules in histiocytes. Lipoid material has been reported by many but not all workers using both frozen and paraffin-embedded tissue. There is unanimity as regard the absence of metachromasia and anisotropism and several authorities have demonstrated the presence of protein material.

The limited studies on the present material produced results which are in general accord

Author	Age	Sex	Symptoms	Site	Characteristics	Size	Treatment	Other information
Abrikossof 1931 (Case 3)	24	♂	Chronic hoarseness	Free edge of Posterior third of Right vocal cord	Red tumour	Small pea	Local removal	Hospitalised for treatment of Trachoma
Dawydow 1931 32	30	♂	Hoarseness for 1 yr Slight dyspnoea and throat pain for 1 m	Free edge of Posterior & Middle thirds of Right vocal cord	Whitish coloured	Large pea	Incomplete local removal	Swollen false vocal cords Slight indentation opposite area of Left vocal cord Laryngofissure refused
Derman & Golbert, 1931 (Case 2)	30	♂	Slight respiratory difficulty for 1 yr and throat pain	Free edge of Right vocal cord	Dull white colour	Lentil	Incomplete local removal	2 whitish, firm, nodular fragments, size of lentil or half a pea removed. Further treatment refused
Glasunow 1933 (Case 2)	38	♀	Hoarseness for 1 m	Under Left ocal cord		Cherry	Local removal	Post-operative radiotherapy
Gechelin, 1934	40		Hoarseness and unpleasant sensation	Posterior half of Left vocal cord, extending onto its upper and lower surface	Pink coloured	1½ cm diam. Pea	Cautery to base Local removal	
Kleinfeld, 1934 (Klemperer 1934 C se 3)	50	♂	Hoarseness for yr Occasional dyspnoea and syncope	Junction of middle and anterior thirds of Left vocal cord	Whitish, nodular	1 cm diam. approx.	Local removal	Klemperer records the case as showing involvement of Right vocal cord
Kerna & Cracovaner 1935	21		Hoarseness for 3 m	Posterior two-thirds of Left vocal cord	Friable rough tumour		Local removal	
Bobbio, 1936	33	♂		Left ocal cord	Polyp	Grain of hemp		
Frenckner 1938 (C se 1)	25	♂	Swelling in throat of sudden onset. Occasional difficulty i breathing	Left arytenoid region	Round, nodular slightly red	Walnut 3½ ½ cm	Local removal	Case in trachea
Iglauer 1942	41	♀	Hoarseness for 18 m	Posterior portion of larynx between arytenoid cartilages	Large, pedunculated globular butter colour	Hazelnut	Local removal	
Maguda & Young, 1953	33	♂	Symptomless	Vocal process of Left vocal cord	Whitish glistening nodule	0.3 0.3 0.4 cm	Local removal	
Somers & Farinacci, 1953 (Case 1)	63	♂		Thickening of Left vocal cord Small nodule near arytenoid	Leucoplakic looking		Local remov l	Previous pulmonary tubercle sputum negative at time of GCM 1 yr later developed thickening. Right vocal cord – TB
— (Case 2)	4	♂	Hoarseness for 2 yr	Left ocal cord near the arytenoid	Pearly lesion	1.5 cm	Removed by laryngofissure	Previously treated for Syphilis 6 yr before Also chronic glomerular nephritis
MacN ughton & Fraser 1954	9	♂	Huskiness for 2 y Slight tridor for 4 m	Inter t noid region xtending dow posterior wall of Trachea	Very granular		Biopsy Laryngofissure Irradiation (3 250 R)	Histology indefinite initial remov l inadequat wi hout ffect

[illegible]ay, 1954	44	♂	[illegible] Cough for some years	[illegible] process		[illegible]	[illegible]	
Keohane, 1956	54	♀	Hoarseness of recent onset	Posterior third of Right vocal cord	Firm, fleshy sessile	5 mm approx.	Local removal	Old inactive bilateral pulmonary tubercle
Hinton & Weinberger 1958	31	♂	Hoarseness and dysphagia for 3 m	Superior t Left false cord and just anterior to Left arytenoid cartilage	Firm, spherical	0.5 cm pprox.	Local removal	Previously treated for Syphilis 11 yr before. Vocal nodules lso seen but removed 15 m later
Bertogalli, 1959	47	♂	Hoarseness for 1 yr	Edge of Left vocal cord	Regular polypoid, sessile mass. Whitish-grey colour	Half a coffee bean	Local removal	
Sylwanowicz & Miodunnewska, 1959	40	♀	Hoarseness for 2 yr	Posterior third of Right vocal cord		Large broad bean	Local removal	
Babbs, 1960 (Case 1)	27	♂	Hoarseness for 6 m. Slight cough	Middle third of Left vocal cord	Papilloma-like mass		Biopsy Laryngofissure	Entire Left vocal cord removed. Nodular area 5 mm diam. below raised central portion
— (Case 2)	53	♂	Hoarseness for 7 wk	Edge of Left vocal cord posteriorly	Small rounded lesion		Local removal	
— (Case 3)	34	♂	Hoarseness for 6 m. Minimal associated dysphagia	Inner aspect of Right arytenoid	Cystic mass		Local removal	
— (Case 4)	32	♂	Strangling cough for 1 wk	Left arytenoid	Mass		Local removal	
— (Case 5)	45	♂	Hoarseness for several months	Left vocal cord	Large polyp		Cord stripped	Vocal cord polyp removed from posterior surface of Right vocal cord several days later
Beckhuis 1960, (Case 1)	45	♂		Posterior third of Left vocal cord, extending into ventricle and into inter arytenoid area.	Superficially cerating, raised lesion		Local removal	
— (Case 2)	39	♂	Marked hoarseness for 3 wk	Posterior third of Right vocal cord extending int inter-arytenoid space.	Greyish-raised plaque		Local removal	Area of leucoplakia towards anterior commissure.
— (Case 3)	39	♀	Recent sore throats Hoarseness for 1 yr	Free edge of Posterior third of Right vocal cord	Papilloma		Local removal	Similar minor changes on adjacent area of opposite cord
Walter 1960 (Case 1)	60	♂	Hoarseness & dyspnoea for 2 m	Neck of laryngocoele sac of Left laryngeal ventricle	Soft, tan nodule	1 5 1.5 1.0 cm		GCM located t Left lateral laryngotomy oper tion t remove laryngocele Operation preceded by tracheostomy 3 wk previously
— (Case 2)	31	♂	Hoarseness of 15 yr duration	Anterior third of Left vocal cord	Large pedunculated pinkish-grey lesion		Local removal	
Chieppe, 1961	31	♂	Progressive hoarseness for 5 yr Dyspnoea on effort 1 yr	Beneath the posterior third Left vocal cord extending to the Arytenoid	Dark red colour Pedunculated with broad base		Too h rd for local moval	Removed through laryngofissure pproach. Coagulation t base [illegible] m

Author	Age	Sex	Symptoms	Site	Characteristics	Size	Treatment	Other Information
Lyons, Haindel & Hiatt 1962 (Case 1)	30	♂	Hoarseness for 3 m	Right vocal cord	Nodular mass	3 mm	Local removal	
— (Case 2)	31	♂	Harsh voice for 8 yr	Junction of middle and posterior thirds Right vocal cord	Polypoid	2 mm	Local removal	
— (Case 3)	34	♂	Symptoms of pneumonitis only	Posterior commissure	Firm red mass	3 mm	Local removal	Similar 5 mm mass removed from base of tongue
— (Case 4)	69	♀	Intermittent and progressive hoarseness for 3 yr	Posterior third of Left vocal cord extending into Posterior commissure	Sessile flat mass	1 cm	Repeated biopsies showing hyperplastic squamous epithelium	Mass 7 5 4 mm removed by hemilaryngectomy through laryngofissure approach
(Case 5)	32	♂	Acute laryngeal obstruction	Posterior third of Right vocal cord extending into Ventricle	Bulbous, smooth red mass, non-ulcerated	2 3 cm	Tracheostomy & biopsy	Followed by hemilaryngectomy through laryngofissure approach Complicated by formation of scar tissue which required excision and irradiation
Ward & Osborn, 1963	41	♂	Slight amount of intermittent hoarseness	Near Vocal process of Right vocal cord	Pearly-grey whitish, nodular sessile lesion	1½ 2 mm	Local removal after biopsy	
Gosavi & Bond, 1964	12	♂	Hoarseness and occasional dyspnoea	Right vocal cord	Pedunculated	Large pea	Local removal	
Halperin, 1964	23		Frequent sore throat for 3 yr	Posterior third of Right vocal cord with subglottic extension	Large pale irregular nodular mass		Local removal	
Ottosson, 1964	9	♂	Hoarseness for past few months	Sinus of Morgagni	Pale excrescence	Grain of rice	Local removal incomplete	Followed 1 m later by laryngofissure and 3 m later by total laryngectomy Massive growth infiltrating laryngeal surroundings and extending into oesophagus
Westernhagen, 1964	30	♂	Slight hoarseness for some years	Posterior third of Left vocal cord	Broad based tumour with almost smooth surface	Cherry	Local removal	
Pope 1965 (Case 1)	2		Sensation of having swallowed a fish-bone	Posterior surface of Right arytenoid	Rounded pink mass	8 mm	Local removal	
— (Case)	3	♂	Hoarseness for 5 m	Posterior aspect of Right vocal cord	Polypoid mass	1 cm	Biopsy	Followed by laryngofissure and partial cordectomy
(Case 3)	37		Hoarseness for 2 wk	Posterior part of Right vocal cord	Smooth pink nodule	5 mm	Local removal after biopsy	
— (Case 4)	14		Asymptomatic	Posterior part of Right	Pale fusiform	[illegible] mm	Local removal	

[illegible]accourreye, Le Gland & Coun, 1966	64	♂	Hoarseness for 1[illegible] m	[illegible] rising from the floor of Right ventricle	[illegible] red	[illegible]	[illegible] cordectomy-anterior part of Right vocal cord, including floor of ventricle and part of thyroid la. Tracheostomy	
Cracovaner & Opler 1967	10	♀	Hoarseness for 6 m	Anterior commissure below vocal cords and attached to undersurface of both cords	Pale pink, irregular nodular smooth, firm tumour	1.5 cm	Local removal Right side Left side 3 m. later	
Sodee, 1967	43	♂	Hoarseness for 6 wk	Middle of Right vocal cord	Firm, semi-sessile polyp	2 3 mm	Local removal	Previous symptoms 4 yr before Polyp removed. No histology
Guerrier Dejean, Galy & Serron, 1968	82	♀	Acut episode of laryngeal stridor which ceased spontaneously without cyanosis. Similar episode the night before. Hoarseness arising over previous 5 yr	Left ventricula band	Greyish-red, pedunculated mass	Almond	Local removal	
Otto & Rose, 1968	27	♂	Dryness in throat. Stabbing pain in larynx, Rough, somewhat hoarse voice	Free edge of Left vocal cord	Whitish, firm, small based, tumour	3 mm pprox.	Local removal	
Piquet, Bloody Leduc, & Decroix, 1969 (Case 1)	30	♀	Progressive hoarseness for 6 m	Anterior third of Right vocal cord	Large polypoid mass		Local removal from Right cord, followed 6 days later by removal from Left cord.	Histology of only one tumour given
				Middle third of Left vocal cord	Smaller polypoid mass			
— (Case 2)	28	♀	Acute laryngeal stridor Progressive hoarseness for 1 yr	Posterior of Left vocal cord	Red coloured, slightly irregular pedunculated	1 cm	Emergency tracheostomy Local removal of tumour	
— (Case 3)	50	♀	Hoarseness for 2 m Progressive dysphagia for 6 m Dyspnoea for 2 wk	Oedema of Left arytenoid heaping up of Left pyriform fossa. Fixed hemilarynx	Mass beneath mucosa of pyriform fossa		Tracheostomy Low l teral pharyngotomy with resection of [illegible] terior part of thyroid ala	Tumour extended under mucosa pushi g pyriform fossa inwards along the inter-cricoid membrane. Tumour excised
Serdana & Yadav 1969	46	♀	Progressive dysphagia for solids for 11 m	Right arytenoid cartilage and posterior cricoid region	Pale growth with broad base	Chestnut	Local removal	
Schneider Gould, & Mirand, 1969 [illegible]ase 1)	31	♀	Persistent hoarseness for 5 yr	Posterior quarter of Left vocal cord, Left arytenoid area & inter-arytenoid space	Mass		Biopsy	Followed by removal by laryngofissure. Tracheostomy

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Author	Age	Sex	Symptoms	Site	Characteristics	Size	Treatment	Other information
— (Case 2)	37	♂	Persistent hoarseness for 6 yr	Middle of Right vocal cord	Granulomatous lesion		Local removal	
Cannalis & Cohn, 1970 (Case 1)	52	♂	Hoarseness for 6 m. Pain in Right side of throat.	Right aspect of Posterior commissure.	Smooth surfaced, white nodule	0.5 0.5 cm	Local removal	
— (Case 2)	68	♂	Hoarseness and cough for 8 m	Posterior third of Left vocal cord	Gray sessile mass		Local removal	
— (Case 3)	65	♂	Mild hoarseness	Middle third of Right vocal cord	Smooth with nodule	0.4 0.4 cm	Local removal	"Wart on larynx first noticed 2½ yr earlier
— (Case 4)	57		Symptomless	Posterior third of Left ocal cord	Polypoid mass	0.3 0.3 cm	Local removal	Hyperthyroidism
— (Case 5)	37		Hoarseness for 3½ yr Stridor on examination	Right arytenoid area	Large yellowish, globular	2.5 1.0 cm	Local removal	

with the foregoing account. There was a varying degree of positivity with PAS staining which was not altered by diastase Alcian Blue staining was weakly positive and no metachromasia or birefringence could be demonstrated in the granules. The failure to demonstrate lipoid material in paraffin sections of 3 out of 4 cases and a protein component in 1 case out of 2 would seem to be no more than a reflection of the varying composition of these granules. Until more is known regarding their nature and formation it is clearly impossible to draw any conclusions on individual differences The results of the more extensive investigations possible in Case 5 are consistent with the current view that the granules may contain glycoprotein and lipoid material whilst the presence of polysaccharide is controversial

The strongly PAS-positive particles found in the non-granular cells in Cases 2, 3 and 5 but not in Case 4 are clearly the angular bodies" first described by Bangle (1952) They were observed subsequently by Fisher & Wechsler (1962) who noted their intensity of staining with Schiff reagent. In Fig. 4 these bodies are illustrated as predominantly rod-shaped but in other cells they assumed a rounded or polygonal form. Whilst they are seen mainly in what may be termed interstitial cells, some of the larger particles in the granular cells may be of a similar nature Certainly their behaviour with PAS stain is identical suggesting that all contain 1 2 glycol groups. A recent report on their fine structure (Aparicio & Lumsden, 1969) suggests that in both situations they are composed of aggregations of microtubules. Although their precise significance is so far unknown, it is clear that the angular bodies are a feature to be looked for in this lesion.

The electron microscopic picture in granular cell myoblastoma is very characteristic. The cells are usually packed with an assortment of pleomorphic granules and vacuoles, often to the exclusion of any other recognizable plasma component. The impression is gained of extensive disorganization and degeneration in

which the nucleus remains unaltered Ultrastructural studies are in progress on the re currence" in Case 4 and will be reported else where. Preliminary studies have revealed a pic ture similar to that found in granular cell myoblastomas occurring in other anatomical sites

There are no clinical features by which the surgeon could recognize granular cell myoblastoma in the larynx and the diagnosis has to await the histological examination, usually on paraffin-embedded material which facilitates diagnosis but limits further study The essential features are faintly eosinophilic and variably PAS-positive granular cells which are of variable shape and show no particular arrangement, though occasional nests or columns are observed. Blood vessels and interstitial connective tissue are usually minimal but interspersed angular body" cells may be discernible, more particularly by virtue of their intense PAS-positive staining. Granular cell lesions of any type are very uncommon in the larynx hence the distinction from such conditions as granular histiocytic lesions, alveolar soft part sarcoma or oxyphil granuloma (oncocytoma) would rarely have to be made It should be axiomatic that carcinoma is never diagnosed in the presence of this granular cell lesion. The aetiology of this mysterious tumour will clearly have to await a more detailed assessment of the wealth of information which is now accumulating, particularly as a result of electron microscopical studies.

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ZUSAMMENFASSUNG

Es werden fünf neue Fälle von gekörnt-zelligem-Myoblastom (Myoblastenmyom) des Kehlkopfes berichtet. Ein Fall betraff einen Jungen von 12 Jahren. Eine Frau von 36 Jahren erlitt ein Rezidiv des Geschwulstes nach 14 Jahren. Es wurden das histologischen Falls beschrieben. In drei Fällen ist es uns gelungen, die sog. „angular body cells (Bangle) zu demonstrieren. Histochemische Untersuchungen ergaben typische Ergebnisse. Es wurden die Theorien der Aetiologie erörtert. Wir fanden 64 Fälle von gekörnt zelligem-Myoblastom des Kehlkopfes in der Literatur 4 Fälle schienen unvollständig bearbeitet zu sein. Die Gesamtheit der veröffentlichten Fälle (69) bildet 10% aller Falle des gekornt zelligen-Myoblastoms. Die meisten Larynx-tumor (gekornt zelliges-Myoblastom) gab es im Alter von 30 bis 40 Jahren und überwogen bei Männern mit 2 1

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THE NORMAL VARIATION OF THE PAROTID SIZE

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Abstract The individual variation of the parotid size was studied by sialography on 9 healthy subjects of both sexes at an age of 26 to 64 years. It was shown that the variation between the individuals in the size of the parotid glands was very large and that the distribution of the values was approximately normal. In diagrams are shown the upper and lower limit for the size of the parotids according to the sigma rule The correlation between the extension of the gland in sagittal and frontal planes was studied, likewise the correlation in size between the right and left sides. The high correlation between right and left sides showed that a sure difference in size between the right and left glands should be regarded a abnormal. The connection between some basic variables and the size of the parotid glands was also studied. There was a weak but significant correlation between sex, body weight, year of life gynecological state and the glandular size.

It is well known that asymptomatic parotid swelling is an important medical finding which in a considerable number of cases can disclose more severe underlying diseases or conditions In other words, symptomless enlargement of the parotids can be a warning sign implying that further studies are necessary The condition, which appears to involve the salivary glands asymptomatically and the parotids in particular is often associated with sarcoidosis, liver cirrhosis and fibrosis, alcoholism, gout, nutritional deficiency toxic disorders (Rothbell & Duggan, 1957 Borsanyi & Blanchard, 1961 Borsanyi, 1962, 1963 Greenberg et al 1964 Goobar & Goobar 1965 Seifert, 1965) or according to some authors, nervous conditions (Seifert, 1967 Pohto, 1966) Asymptomatic enlargement is also seen in endocrine disturbances, especially diabetes mellitus, thyroid disease and menopausal states (Borsanyi, 1962 Angervall et al., 1963 Diamant, 1966 Davidson et al., 1969)

However it is difficult to diagnose swellings of the salivary glands, as only obvious enlargement can be palpated. Moreover the palpation gives very poor information in distinguishing between swelling of noninflammatory origin from inflammatory or tumor swellings Special difficulties are connected with the diagnosis of slowly developing swellings, chiefly of the parotid gland These swellings often occur without other symptoms, and, as a rule other methods of examination are negative (Diamant & Forsberg, 1959 Blatt, 1965 Diamant, 1966, 1969) Furthermore no investigation has yet been reported in which the normal variation in the size of the parotid is studied

By using sialography it is possible with a high degree of accuracy to distinguish between noninflammatory and other lesions of swelling and now even to demonstrate minor and moderate changes in the volume of the parotid glands (Garusi, 1963 Einstein, 1966 Pfeiffer 1968 Ericson & Hedin, 1970) It is also possible to follow the degree of change during the course of illness (Henrikson 1970) and to register the size of the parotid glands (Ericson & Hedin, 1970) With a knowledge of the normal range of variation in the volume of the salivary gland determined with sialography and a knowledge of the factors which in healthy individuals affect the size it should be possible to decide with greater accuracy than is attainabl today

whether a gland is pathologically enlarged or not. This may imply increased diagnostic facilities with respect to the salivary glands.

The aim of this paper is to report an investigation where the variation of the parotid glands in healthy people was studied to determine the upper and lower limits of the normal parotid size. The relation between the parotid size and some basic variables will also be reported.

MATERIAL

The material consisted of 92 healthy individuals, 61 females and 31 males, chosen at random within special age-groups from the clientele of the School of Dentistry in Umeå. The age of the 61 women varied between 26 and 64 years, the average being 49.8 years, while the age of the 31 men ranged from 26 to 64 years, with an average of 48.8 years.

The subjects were examined with regard to their general health. Laboratory investigation with respect to ESR, hemoglobin, blood-cell account, syphilis serology, antistreptolysin titre, antistaphylolysin titre, electrophoresis of blood-serum and tests for the rheumatoid factor were performed. No diseases were diagnosed which in the light of current experience gave rise to salivary involvement. None of the women was pregnant at the time of the examination.

The parotid glands were studied clinically and roentgenologically with sialography. All glands were roentgenologically investigated by sialography in the lateral projection. In the frontal projection 11 cases were not investigated. No definite pathological conditions were diagnosed. Three glands from 3 subjects did, however, on the sialograms show an appearance differing somewhat from the normal and from the appearance of the gland of the other side, but here too there was no history of pathological salivary conditions and the subjects themselves were healthy.

For a detailed account of the material the reader is referred to Ericson (1968). This material is identical with the controls in the latter work.

METHOD

For the sialographic examination of the right and left parotid glands, the method described and analysed by Ericson (1968) was used. Th[illegible] central ray was directed towards the m[illegible] of the gland, in the lateral pro[illegible] angle of 78 degrees to the sag[illegible] in the anteroposterior projecti[illegible] to the plane of the film and [illegible] *ramus ascendens of the mandib[illegible]* For the exposures, a precisio[illegible] skull radiography (Elema Scho[illegible] was used. The film to-focus distance wa[illegible] cm, and the contrast medium used was 60% Urografin®. The exposure data were adapted to the patient's constitution by taking primary roentgenograms without contrast medium in the ducts. The injection of the contrast medium was performed slowly and was stopped when the subject felt a moderate pain in front of

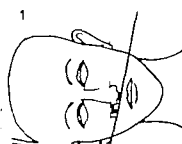


Fig. 1. The orientation of the skull and direction of the beam in lateral projection.



Fig. 2. The orientation of the skull and direction of the beam in frontal projection.



Fig. 3. Sialogram of a parotid gland in lateral and frontal projections. The outer boundary of the gland is traced.

the ear in the cheek. Another 0.1–0.3 ml of contrast fluid was injected and an exposure was made. The amount of injected contrast medium varied but did not exceed 1.2 ml.

For the determination of the size of the gland, the method described by Ericson (1968) and in detail studied by Ericson & Hedin (1970) was used. In th sialograms the parotid gland was outlined with a pencil (Fig. 3) and enclosed area was computed in mm² with Aristo planimeter no. 1130 (Fig. 4). Between this area and the total volume of th gland it has proved to be a very strong relation, r 0.95 (Ericson & Hedin, 1970).

Study of the method error

The errors of the planimetric method were studied (Ericson & Hedin, 1970). The areas of 15 gland as projected on lateral sialograms were traced and calculated by one observer on two different occasions. The range of the area varied between 10.2 20.4 cm and the standard deviation for a single determination was determined to be 0.28. The values for the frontal projection were of the same order of magnitud. That means that the error of measurements including the error incurred in the graphic outlining of the gland was small compared to the variation in size between the individual glands.

The discrepancy between two observers concerning the planimetric determination of the area of the gland as projected in the lateral and frontal sialograms were measured. There were no systematic differences between th investigators. From the high correlation coefficient, $r = 0.92$ it was evident that planimetric determinations of the projection of the parotid gland on sialograms can be performed to a high level of precision.

With the purpose of analysing the extent to

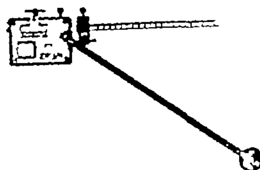


Fig. 4. Aristo planimeter no. 1130 used for the calculations of the glandular size in the sialograms.

Table I. *The mean value ($\bar{x}$), the standard deviation (S.D.) and the lowest and highest value for the area of the parotid gland in the sialogram in a lateral projection (n = 92).*

Side	$\bar{x}$	S.D	Range
Right gland (mm²)	1 55[illegible]	70	990–2 100
Left gland (mm²)	1 560	289	590– 100
Right and left glands (mm²)	3 112	340	1 580–4 190
Right minus left	−8	177	

Table II. *The mean value ($\bar{x}$), the standard deviation (S.D.) and the lowest and highest value for the area of the parotid gland in the sialogram in a frontal projection (n = 81)*

Side	$\bar{x}$	S.D.	Range
Right gland (mm²)	1 619	43	1 010– 180
Left gland (mm²)	1 585	288	420–2 070
Right and left glands (mm²)	3 204	500	420– 180
Right minus left	34	180	

which the projected area is affected by moderate variations in the orientation of the gland in the path of the beam, a comparison was made of three sialograms taken in lateral view one in each of three projections ±3°. In all 45 sialograms from 15 subjects were studied. A discrepancy of six degrees in the angulation during the exposure did not give a significant change of the size of the projected area of the gland.

To sum up the methodological errors due to tracing, calculation and orientation of the objects in the path of the beam were small and negligible compared with the inter-subject variation in size.

For a detailed account of the method and of the errors of the method the reader is referred to the works mentioned above (Ericson, 1968; Ericson & Hedin, 1970).

RESULT

In *Table I* are presented the mean value, the standard deviation of the mean value and the range for the extension of the parotid glands as projected in the lateral sialograms. In Table II the corresponding values for the area of the glands as projected in the frontal sialograms.

From the Tables I and II, it appears that the variation between the individuals in the size of the parotid glands is rather large. Graphically the distribution of the glandular size is shown in Diagrams 1–2. The diagrams show that the distributions of the values assume a rather symmetrical picture; the skewness is moderate and does not deviate markedly from normal distribution. The difference between the mean value and the median value for each sample is not significant. On the right side the median and mean values are almost identical. On the left side one gland diverges extremely in size from the other glands.

In order to be able to determine in the individual case whether or not the size of the gland may be regarded as normal, the deviation around the mean values according to the 2 sigma rule is pointed out in the diagrams. Within these limits 95% of the population should lie. In Diagram 1 five glands of 184 are outside these limits; in Diagram 2, six glands of 162.

The relation between the extension of the right and left glands in lateral and frontal projections is given in Table III. From the table,

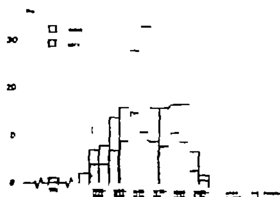


Diagram 1. Distribution of the size of the right and left parotid glands in lateral projection. Mean value and ±2 S.D. is marked.

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THE ULTRASTRUCTURE OF THE HUMAN STRIA VASCULARIS. PART II

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Abstract. A study was made of the pathological findings in the stria vascularis obtained from patients at the time of labyrinthectomy. Electron microscopy reveals structural alterations in the cells which would not have been obvious by light microscopy. All three types of cell are affected: the earliest and most frequent changes are found in the marginal cells. Atrophy is common; the most frequent sites are near the spiral prominence and near Reissner's membrane. In severe atrophy the basal cells align the endolymphatic surface. In a few specimens the marginal and intermediate cells show unusually high metabolic activities. A few highly vesiculated cell processes of undetermined origin are found in the deeper part of the stria vascularis. The intermediate cells and some cells in the spiral ligament show the morphological characteristics of secretory cells. There is no evidence of viral particles. Blood vessels are no different from those of the stria vascularis of non-Menière specimens. It cannot be stated with certainty that these findings, while representing definite pathological alterations, are related to Menière's disease as we do not yet know the norm for the ageing human ear.

We obtained the stria vascularis in an excellent state of preservation from the basal turns of human cochleae at the time of labyrinthectomy for unilateral disabling Menière's disease. In a previous study (Kimura & Schuknecht, 1970) we described the ultrastructure which we considered to be normal when compared with well-preserved animal specimens and human post-mortem specimens. The purpose of the present study is to describe the changes in ultrastructure observed in the same specimens.

The over accumulation of endolymph in Menière's disease has been well documented (Hallpike & Cairns, 1938; Yamakawa, 1938; Lindsay, 1942; Altmann & Fowler, 1943; Schuknecht et al., 1962). Although the cause of the increase in endolymph volume has not been clearly determined, it seems probable that the physiological basis is either decreased resorption or increased secretion. Experiments with animals (Kimura & Schuknecht, 1965; Kimura, 1967; Schuknecht et al., 1968) have shown that endolymphatic hydrops may be produced by ablation of the endolymphatic sac which presumably has a resorptive function. The stria vascularis assumes importance because it is considered to be the site of endolymph production in the cochlea (Guild, 1927; von Fieandt & Saxén, 1937; Altmann & Waltner, 1950).

In this study we will discuss the significance of structural alterations in relation to Menière's disease. The technical details are given in our previous report; specimens were fixed in 1% phosphate-buffered osmium. The ten specimens examined are distributed in the following age groups: 2 between thirty and forty, 5 in the fifties, and 3 between sixty and eighty.

FINDINGS

Endolymphatic hydrops is demonstrable in the small pieces of the labyrinths, even after surgical removal (Fig. 1). Reissner's membrane at its lateral attachment is elevated more toward the scala vestibuli in a manner similar to that described in light microscopic studies. Reissner's membrane may appear normal at this point even though the stria vascularis in the

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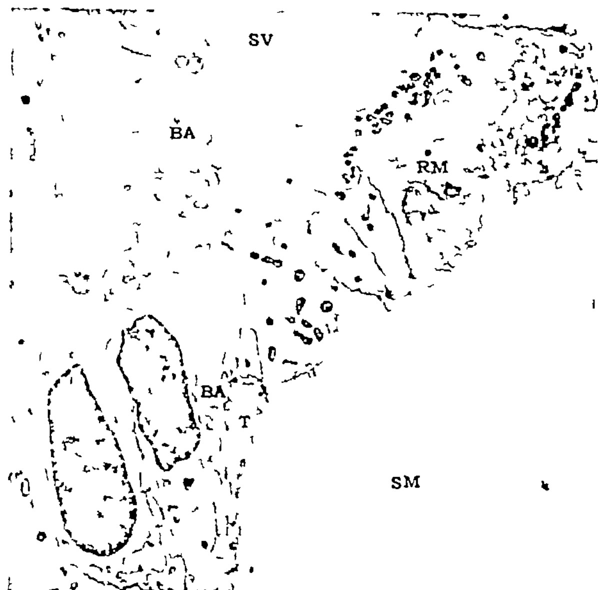


Fig 1 Attachment of Reissner's membrane (*RM*) to the lateral wall of the cochlear duct. The position of Reissner's membrane without obvious distortion or mechanical injury to the cells at this site suggest a hydrops in the intact cochlea. Reissner membrane here appears to be normal, but the stria vascularis (see Fig. 7 C) is severely atrophic. A cluster of tightly packed cell processes adjacent to the scala vestibuli (*SV*) is a continuation of the basal cell (*BA*). *SM* Scala media. *T* transitional epithelium. 5 600. Age 39; duration of Menière's disease 3 years.

immediately adjacent area shows severe atrophy (Fig. 7 *C*).

In almost all specimens, structural alterations are observed in some parts of the stria vascularis in contrast to light microscopic studies which have consistently demonstrated this structure to appear normal. In general, as the thickness of the stria vascularis decreases, the extent of pathology increases. Atrophy may be localized or diffuse. A limited lesion can occur anywhere but the most frequent sites are at the margins of the stria, i.e. near the attachment of Reissner's membrane and near the spiral prominence, the latter being the most frequent site. The thickness of the epithelium is abruptly reduced when the blood vessels and intermediate cells disappear, leaving flat marginal cells. When the thickness of the epithelium de-



Fig. 2. (*A*) Atrophic marginal cells showing decreased number of pinocytotic vesicles, RNA particles and granular endoplasmic reticula; the mitochondria show an increased matrix density. ×18 000. Age 40; duration of Menière disease 5 years. (*B*) The marginal cells are filled ... replace other cell or... high magnifi... in cross sectio...

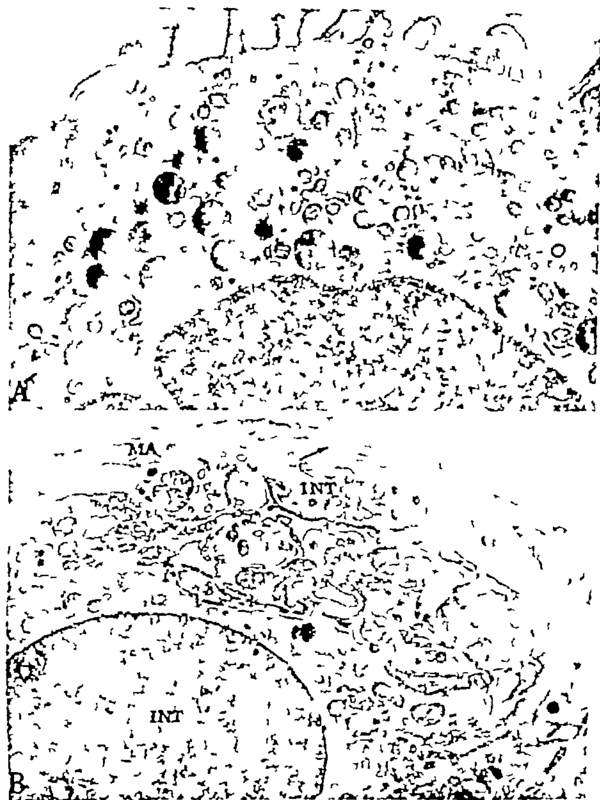


Fig 3 (*A*) Marginal cell containing numerous round granules, presumed to be lysosomes. × 7 000 Age 53 duration of Menière disease [illegible] years. (*B*) The marginal cell is thin and is invaded by cell processes of the intermediate cell (*INT*) Note [illegible] short basement membrane (arrow) and a wide intercellular space. × 13 000 Age 61 duration of Menière [illegible] 10 years.

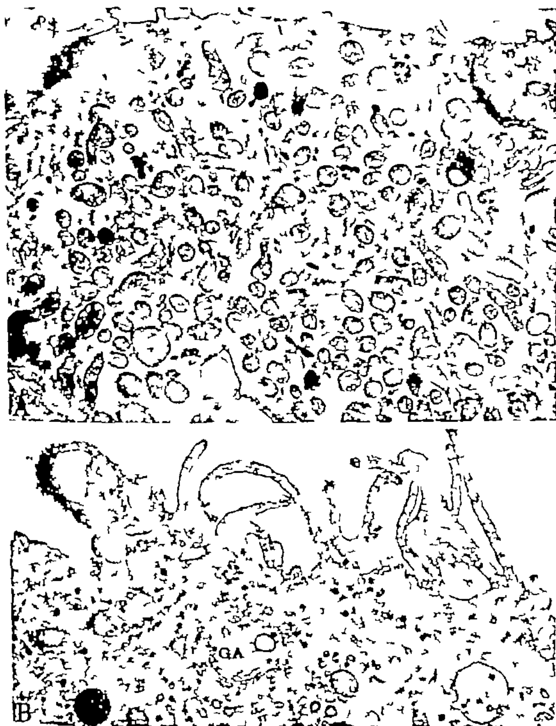


Fig. 4. (*A*) A marginal cell showing unusually numerous mitochondria at the apical zone. ×24 000. Age 40, duration of Menière's disease 15 years. (*B*) A marginal cell showing unusually tall microvilli. *GA* Golgi apparatus. ×29 000. Same specimen as in Fig. 3 *A*.

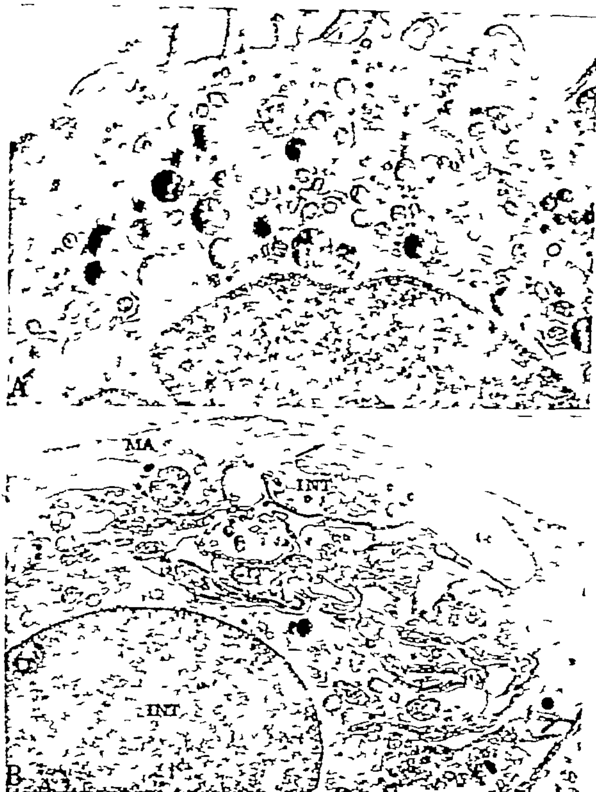


Fig. 3. (*A*) Marginal cell containing numerous round granules, presumed to be lysosomes. ×7 000. Age 53, duration of Ménière's disease years. (*B*) The marginal cell is thin and is invaded by cell processes of the intermediate cells (*INT*). Note the short basement membrane (*arrow*) and a wide intercellular space. ×13 000. Age 61, duration of Ménière's disease 10 years.



Fig. 5. The marginal cell shows long mitochondria containing longitudinally arranged cristae at the infranuclear zone (*arrows*) and short mitochondria with transverse cristae at the apical zone. 16 000. The inset shows high magnification of the long mitochondria and granular endoplasmic reticula taken from the adjacent section. Same specimen as 3 B



Fig 6 (*A*) Strial atrophy The marginal cell (*MA*) becomes flat and shows a decrease in cell organelles and intercellular interdigitations. The intermediate cells (*INT*) are relatively unaffected. The dark layer over the marginal cells is a precipitate resulting from the surgical removal of the specimen. *BA* Basal cell, *L*, lipofuscin granule. 5 500 Same specimen as in Fig. 4 *A* (*B*) The intermediate cell (*INT*) contains numerous inclusion bodies, presumed to be lipofuscin granules. *MA* Marginal cell. 11 800. Same specimen as in Fig. 4 *A*

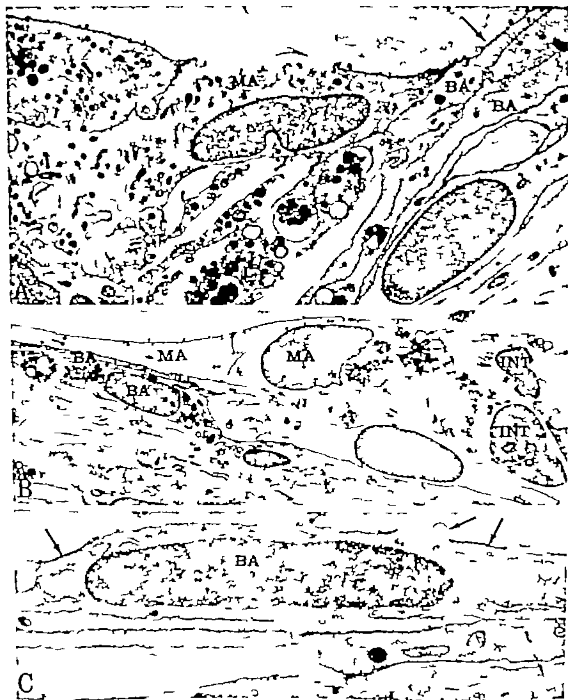


Fig. 7. (*A*) An extension of the basal cell (*BA*) to the luminal surface in the area between the marginal cell (*MA*) and the spiral prominence. *L*, Lipofuscin granules. 7 00 Same specimen in Fig. 3 *B*. (*B*) Strial atrophy. The marginal cells (*MA*) are flat and are directly abutted by the basal cell (*BA*). *INT* Intermediate cells. 4 400. Same specimen as in Fig. 4 *A*. (*C*) Severe strial atrophy. Basal cell (*BA*) is directly exposed to endolymph. Cell processes of atrophic marginal or intermediate cells (*arrows*) are still identifiable. 20 000. Same specimen as in Fig. 1

underlining the atrophic marginal cells (Fig. 7 *B*) Sometimes the basal cells disappear leaving flat cells, presumably the marginal cells, underlined by a continuous basement membrane *On the other hand* when the marginal and intermediate cells disappear the basal cells may remain intact In such cases the basal cells emerge to the endolymphatic surface (Fig. 7 *C*) The basal cells often show prominent lipofuscin granules and their cytoplasmic filaments may increase, though such filaments are rather common in the normal cells.

The capillaries of the stria vascularis often show no obvious structural change even when the marginal cells demonstrate alterations, but in a later stage they appear to atrophy concommitantly with other strial tissue. Some of the changes seen in the endothelial cells of the capillaries are the presence of many lysosome granules (Fig 10 *C*), huge masses, and some increase in the number of filaments. The large masses contain a homogeneous, opaque substance surrounded by a light band of varying thickness showing a series of parallel lines numbering up to seven. The shape of such masses is oval or half of the oval in profile (Fig. 10 *A* *B*) Pericytes around the endothelial cells are ...us, but may overlap Blood vessels surrounded by a thick layer in which are a ... of basement membranes interspersed with ...ine fibrils and, more peripherally thick fibers some of which demonstrate cross striations of the collagen type Connective tissue elements increase in the perivascular area. When the capillary walls are disintegrated, the area of the lumen is filled with fibrils, homogeneous substance, and some cell processes of undetermined origin

In the spiral ligament adjacent to the stria vascularis there frequently are large, striated bodies among the fibrocytes (Fig 11) These bodies are composed of a series of alternating light (460 A) and dense (500 A) bands in parallel Within the dense band is a slightly lighter narrow band. All dense bands appear interconnected with fine filaments. The bands of these striated bodies change direction and the dense bands become continuous with a homogeneous mass of equal density They are not membrane-bound, and adjoin intercellular fibrils and fibrocytes In one specimen, an unusual cell type was found It contains numerous smooth endoplasmic reticula some of which show annulate lamellae, a few localized narrowings of opposite membranes where cytoplasmic condensations are noted (Fig. 12)

DISCUSSION

The present investigation reveals that the stria vascularis removed from patients afflicted with Menière s disease shows normal, atrophic and some hyperactive cells. The stria may appear normal under the light microscope but is atrophic under the electron microscope Among the strial cells the earliest and the most frequently affected are the marginal cells which are thought to engage in fluid transport activity In mild atrophy the thickness of the stria may not change In an advanced stage both the marginal and the intermediate cells are involved with any one of these cell groups missing. In general the severity of the change is correlated with the thickness of the stria vascularis the thinner layer indicates more atrophy Predilection of atrophy is apparent the peripheral zones of the stria vascularis near the spiral prominence and near the Reissner s membrane are most often affected. The reason for the vulnerability of these areas is not clear but there may be some relation to a paucity of vascular supply In contrast to this localized nature, atrophy can be diffuse throughout the strial width in which case the marginal cells are most severely affected.

The cells of the transitional zones are different from the marginal cells and the epithelial cells of Reissner s membrane Evidence suggests that the transitional cells are closely related to the basal cells (Figs. 1 7 *A*) When the marginal and intermediate cells degenerate, they are often replaced by the transitional cells and basal cells, thus maintaining a barrier between the endolymph and perilymph spaces (Fig. 7 *C*).

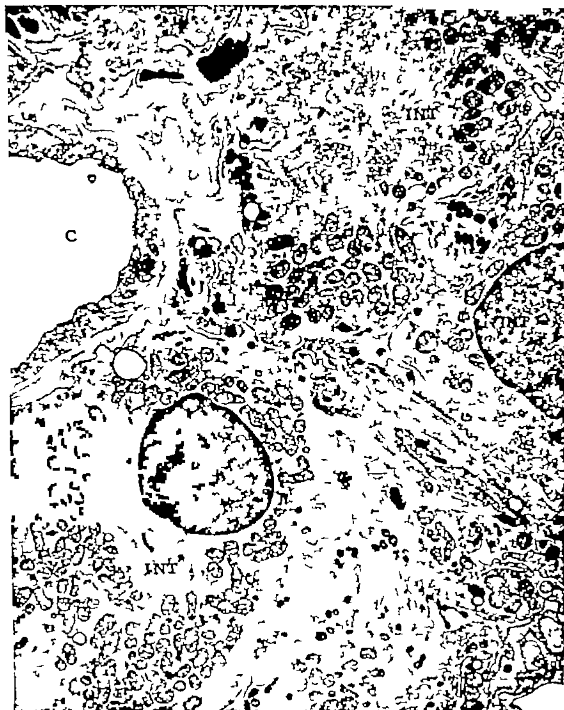
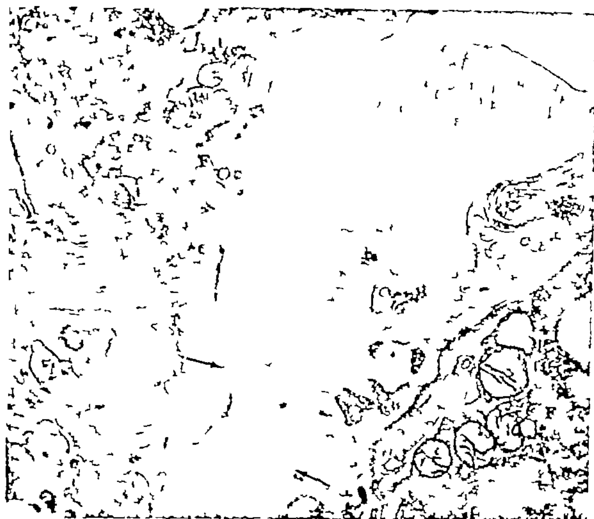


Fig. 8. The intermediate cells (*INT*) demonstrating an unusually large number of mitochondria at the lower left, and numerous smooth endoplasmic reticula and Golgi apparatu t the upper right corner. The intermediate cells in lose sociation with the cap lary (*C*) 7 500. Same specimen as in Fig. 4 *A*.



11. Striated bodies found among the fibrocytes in the spiral ligament. Some areas (*arrows*) appear in a stage of developing the striated body. Direction of parallel bands changes abruptly almost at right angles. × 28 400. Age 52; duration of Menière's disease 10 years.

8 kc range. Strial atrophy of the ageing ear is well documented by Schuknecht (1964). An increase in the intracellular filaments, lysosomes and lipofuscin granules may be related to ageing (Figs. *2 B*, *3 A*, *6 B*). The structural alterations reported here may involve hydrops or ageing or both, and perhaps other factors such as the tears in Reissner's membrane (Duvall, 1968).

In the marginal cell, it is of interest to note that the mitochondria containing longitudinal cristae are located at the infranuclear zone while mitochondria with transverse cristae are found at the apical zone (Fig. 5). A high correlation between the mitochondria with longitudinal *cristae* and the absence or reduction in cytochrome oxidase activity has been found in the proximal kidney tubules of the winter frog (Karnovsky, 1963). Similar mitochondria are observed in the fibrocytes or stroma cells of the spiral ligament; however, they become electron-dense and fuse exhibiting a pleomorphism (Takahashi & Kimura, 1970). In the marginal cell a functional alteration is indicated earlier in the basal zone than in other areas.

In a few specimens the marginal and intermediate cells show the morphological charac-



Fig. 12. A cell in the spiral ligament which is in an active secretory stage. Note numerous smooth endoplasmic reticula, mitochondria and some lipid-like masses. The inset shows high magnification of the smooth endoplasmic reticula at which cytoplasmic condensation is noted at the narrow points (*arrows*) of the cisternae (anaulate lamellae). Arrows in the large photograph show similar endoplasmic reticula. 11 900. Age 55, duration of Menière's disease 10 years.

teristics of high metabolic activity (Figs. 4 A 8). In particular the concentration of mitochondria is much higher than in other human post mortem specimens and fresh animal specimens such as guinea pig, cat and squirrel monkey. These characteristics may represent a normal morphological variation or may have a functional tie in producing hydrops, or may

sent activity of the secretory cells to compensate for endolymph depletion created by atrophy in other parts of the stria (Figs. 6 *A* 7 *B*) These cells, unusually rich in cell organelles, are found in the specimens from patients who had symptoms of Menière s disease for 25 years (age 80), 10 years (age 61) and 14 years (age 52) respectively The presence of these cells in older patients suggests that secretory activity has been maintained at a high level in some parts of the stria, regardless of ageing.

Judging by the presence of numerous smooth endoplasmic reticula and Golgi apparati and a high concentration of mitochondria (Fig. 8), the intermediate cells are a secretory type of cell. In the intermediate cells of animals these elements are not an outstanding feature (Smith, 1957 Hinojosa & Rodriguez Echandia, 1966 Spoendlin, 1967) In other areas of our specimens the intermediate cells show the same morphological characteristics as those of animals and they are considered to be normal (Kimura & Schuknecht, 1970) The precise role played by these cells is unknown. The marginal cells adjacent to these intermediate cells are either normal or show a higher concentration of mitochondria, suggesting that the functions the two types of cell may be related.

Some cell processes in the deeper part of the stria vascularis show numerous cytoplasmic vesicles (Fig. 9) Their main cell bodies are not identified They are unlike typical strial cells but may be a part of the altered intermediate cells. They are often found near the blood vessels. The cells appear to engage in the transfer of fluid or some substances across the cell membrane

No viral particles could be found, and blood vessels are essentially no different from those of non-Menière specimens. Filaments in the endothelial cells vary in number from one group to another and they are more of a supporting type rather than contractile. The presence of huge masses is noted in the endothelial cells of three different specimens (Fig. 10 *A B*) They are unlike secretory granules they may be phagocytized bodies or even altered bacteria. The absence or paucity of smooth muscles in the strial vessels suggests that contraction of the vessels is not a major factor in regulating blood flow within the stria vascularis. Perhaps control is accomplished elsewhere, such as in the spiral ligament adjacent to the scala vestibuli and in the modiolus where smooth muscles are more frequently identified

An unusual cell resembling the fibrocyte in size and shape but different in respect to the number and characteristics of smooth endoplasmic reticula is found in the spiral ligament (Fig. 12) The smooth endoplasmic reticula are extremely numerous and some show an annulate lamellae arrangement (Maul 1970). The cell is apparently in the stage of secretion or differentiation The presence of such active cells suggests some alteration in the fluid and/or cells of the spiral ligament. This cell may be the Type II fibrocyte described in the spiral ligament of the Rhesus monkey by Takahashi & Kimura (1970)

The striated bodies seen in the spiral ligament (Fig. 11) are similar to those described in the vestibular labyrinth removed from Menière patients (Hilding & House, 1964 Friedmann et al., 1965) These striated bodies are called long-spacing collagen" by Ramsey (1965) They are present in the inner ears of non-Menière patients and in other parts of the body The striated bodies are closely associated to fibrils and fibrillar matrix. However the inter connecting filaments between the bands are smaller in diameter than those of the inter cellular fibrils. Fibrils of the spiral ligament are not considered to be collagen in type (Iurato, 196. Hamilton, 1967)

In the present study of the stria vascularis various morphological characteristics are shown among different specimens and even within the same specimen. Atrophy is common but may be a manifestation of ageing or other factors. More information is needed to determine whether the active marginal and intermediate cells, the vesiculated cell processes, and the secretory cells in the spiral ligament are normal

or whether they are specific to Menière's disease. The authors are fully aware of the limitations of this study which is based on segments of the stria vascularis removed from the basal turn of the cochlea.

ZUSAMMENFASSUNG

Wir studierten die pathologischen Verhältnisse der Stria vascularis, die wir von Patienten erhielten, denen wegen Menières Krankheit der Labyrinth entfernt wurde. Im Elektronen-Mikroskop sieht man Änderungen in der Struktur der Zellen, die beim Licht Mikroskop nicht aufgefallen waren. Das betrifft alle drei Zelltypen, aber die ersten und häufigsten Veränderungen sieht man in den Randzellen. Atrophie kommt häufig vor besonders in der Gegend der Spiralen prominence und Reissners Membran. Wo die Atrophie stark ist, richten sich die Basalzellen nach der Endolymphatischen Oberfläche. In einigen Objekten sieht man in den marginalen und intermediären Zellen ungewöhnlich starke metabolische Aktivität. Einige stark vesikulierte Zellfortsätze unbekannter Herkunft befinden sich im tiefen Teil der Stria vascularis. In den intermediären Zellen und einigen Zellen des spiralen Ligamentes sieht man die morphologischen Merkmale sekretorischer Zellen. Man sieht keine viralen Körperchen. Die Blutgefässe unterscheiden sich gar nicht von denen der gesunden Stria vascularis. Obwohl diese Resultate wirkliche pathologische Veränderungen darstellen, können wir nicht mit Gewissheit sagen, dass sie mit der Menières Krankheit im Zusammenhang stehen, weil bis jetzt das normal alternde menschliche Ohr noch nicht auf diese Weise studiert wurde.

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NEW ANATOMICAL ASPECTS OF THE VASCULO-EPITHELIAL ZONE OF THE SPIRAL LIMBUS IN MAMMALS

An Electron Microscopic Study

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Abstract. The ultrastructure of the vasculo-epithelial zone of the spiral limbus of the guinea pig and cat is described. The vasculo-epithelial zone is composed of two types of cells osmiophilic (dark) and osmiophobic (light) cells. The osmiophilic cells line the surface of the vasculo-epithelial zone and are bathed in perilymph. These cells have dense cytoplasm and consist of numerous plasma membrane processes which are filled with tightly-packed mitochondria. Other cellular inclusions comprise an extensive network of endoplasmic reticulum and Golgi apparatus. The inferior layer consists of osmiophobic cells which contain endoplasmic reticulum and mitochondria. Morphologic similarities were noted among the vasculo-epithelial zone and other areas of the inner ear which are capable of active transport, e.g. the mammalian vestibular dark cells and the stria vascularis. The ultrastructural characteristics of the vasculo-epithelial zone suggest that this site is capable of active transport.

The spiral limbus is a complex network of filaments, blood vessels, ground substance and connective cells. The surface of the spiral limbus can be divided into three zones: (1) the zone facing the scala media (tectorial membrane) (2) the zone facing the inner spiral sulcus, and (3) the zone facing the scala vestibuli. Borghesan (1950 1952, 1957) called the latter zone the vasculo-epithelial zone and speculated that this region was capable of secreting perilymph. The spiral limbus in this region consists of connective tissue and connective cells interspersed with capillaries. The epithelium is in direct contact with the perilymph of the scala vestibuli.

Since the classical work of Corti (1851) and Retzius (1884) numerous studies have been conducted to determine the source and absorptive sites of endolymph and perilymph. Cochlear sites such as the spiral prominence (Borghesan, 1957) outer sulcus cells (Shambaugh, 1909 von Fieandt & Saxén, 1937 Duvall, 1969) and Reissner's membrane (Rauch et al. 1963) appear to be involved, in conjunction with the stria vascularis (Shambaugh, 1905 Guild, 1927 Saxén, 1933 Rüedi, 1951 Misrahy et al., 1958), in maintaining the composition and volume of endolymph. It is generally accepted that perilymph is an ultrafiltrate of blood serum from cochlear capillaries, and that the absorption of perilymph is carried out by the capillaries of the spiral ligament (Vosteen, 1963 Vinnikov & Titova, 1964). The communication between the cerebro-spinal fluid and the perilymph suggest that perilymph may also be partly derived from cerebro-spinal fluid via the cochlear aqueduct.

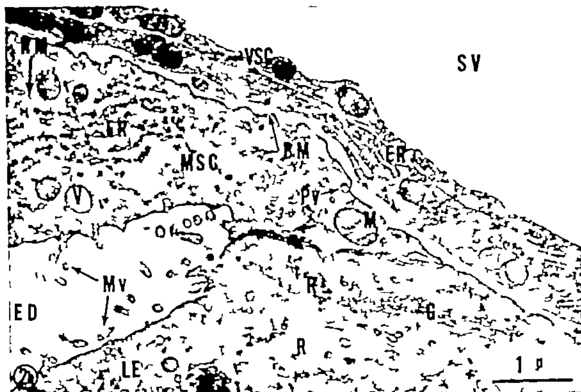
The authors feel that certain networks of cochlear nonsensory epithelia, which were thought to be merely supporting cells, co
be metabolically active and may be cap
local secretion and/or absorption

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Fig 1 Histologic section of the spiral limbus of the guinea pig cochlea illustrating the position of the vasculo-epithelial zone (*VZ*). *IDC* Interdental cells; *IHC* Inner hair cell, *ISC* Inner sulcus cells; *RM* Reissner' membrane; *TM* Tectorial membrane; *Vs*, Capillaries.

Fig 2. (*a*) Electron photomicrograph of the cellular attachment of Reissner's membrane to the spiral limbus of the guinea pig. The basal lamina (*BM*) separates the media squamous cells (*MSC*) and the limbus epithelium (*LE*) from the vestibular squamous cells (*VSC*). The basal lamina continues beneath the limbus epithelium. *M* Microvilli, *VZ* Vasculo-epithelial zone; *RM* Reissner' membrane. (*b*) Electron micrograph of the union of Reissner's membrane and limbus epithelium. The osmiophilic vestibular squamous cells contain endoplasmic reticulum (*ER*) and many mitochondria (*M*). The basal lamina (*BM*) separates the vestibular squamous cells (*VSC*) from both the media squamous cells (*MSC*) and the limbus epithelium (*LE*). The media squamous cells and limbus epithelium are similar in structure. *G* Golgi apparatus; *ED* Endolymphatic space; *SV* Scala vestibuli; *M* Microvilli; *V* Vesicle; *Pv* Pinocytotic vesicle; *R*, Ribosomes; *RM* Reissner's membrane.



epithelial zone, which is of interest in this study is typical of one of these areas. With the exception of Borghesan's light microscopic observations, no published reports were found describing the ultrastructure of the vasculo-epithelial zone. Accordingly this report describes the ultrastructure of the vasculo-epithelial zone of the guinea pig and cat, and hypotheses are presented as to its functional significance.

MATERIAL AND METHODS

Ten healthy guinea pigs (300 to 350 g) and three cats (3.5 to 4.0 kg) were used in this study. Each animal was anesthetized and the bulla was opened widely to expose the round and oval windows. Under the operating microscope the round window membrane was punctured with a fine needle. Using a small dental bur small holes were drilled through the bone of the second and apical turns of the cochlea into the scala vestibuli. Glutaraldehyde (5% glutaraldehyde in 0.1 M phosphate buffer pH 7.35) was introduced into the scala vestibuli with a fine pipette. The animals were decapitated and the temporal bones were quickly removed and refrigerated for 2 hours at 4 C in fresh glutaraldehyde solution. The specimens were then post-fixed with Palade fixative (1952) for 2 hours and dehydrated using routine techniques. Luft's technique (1961) was used to prepare the tissue for Epon embedding. Sections were cut from samples of each turn of the cochlea with a MT 2 Sorvall Porter Blum ultramicrotome and stained with toluidine blue for phase contrast examination. Thin section for electron microscopy were then cut and stained with uranyl acetate (Watson, 1958) and lead hydroxide (Karnavsky 1961). The sections were examined with a JEM 7 electron microscope at 80 kV.

RESULTS

For orientation purposes, the photomicrograph in Fig. 1 illustrates the spiral limbus of the

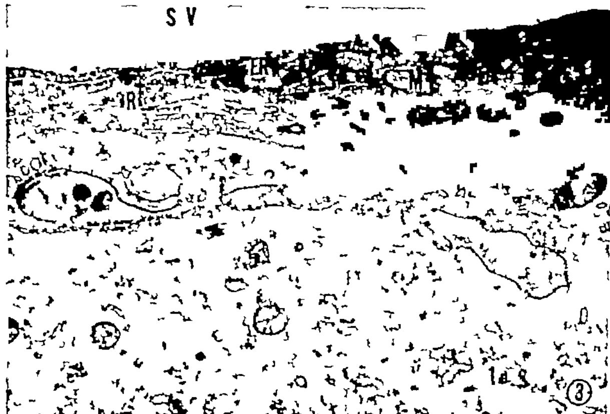


Fig. 3. The spindle-shaped squamous cells have extremely long slender cytoplasmic processes which enclose mitochondria (M) and ribosomes (R). The osmiophobic cells, which are inferior to the osmiophilic cells, contain mitochondria and endoplasmic reticulum (ER). The surface of the plasma membrane which is bathed in perilymph is relatively smooth. SV Scala vestibuli.

nea pig cochlea. The vasculo-epithelial zone Z) is situated between the insertion of Reissner's membrane and modiolus of the cochlea. Medial branches of the spiral artery course through the connective tissue of the limbus and border upon the underlying layers of cells. The electron photomicrographs in Fig. 2 show the cellular attachment of Reissner's membrane to the spiral limbus. As the double-layered Reissner's membrane approaches the superior surface of the spiral limbus (Fig. 2 a) the media squamous cells and the vestibular squamous cells separate from each other. The media squamous cells turn toward and join the outer surface of the spiral limbus (limbus epithelium). The osmiophilic vestibular squamous cells continue along the surface of the scala vestibuli and unite with the vasculo-epithelial zone. The basal lamina, which is present between the media squamous cells and the vestibular squamous cells, continues beneath the cells of the limbus epithelium. The region where Reissner's membrane joins the limbus epithelium appears to be metabolically active (Fig. 2 b). The osmiophobic vestibular squamous cells contain numerous mitochondria and free ribosomes. Endoplasmic reticulum are present throughout both layers of cells.

The vasculo-epithelial zone itself is comprized of two types of cells with different electron densities. The osmiophilic cells (Fig. 3) are spindle-shaped squamous cells with extremely long slender cytoplasmic processes which enclose mitochondria, ribosomes and endoplasmic reticulum. The osmiophobic cells, which are inferior to the osmiophilic cells, contain ribosomes, mitochondria, endoplasmic reticulum and vesicles. These cells, however do



Fig. 4. The relative shapes of the osmiophilic (dark) and osmiophobic (light) cells and their relationship to capillaries are contrasted in this electron photomicrograph. The cuboidal-like light cells containing endoplasmic reticulum (*ER*), and mitochondria (*M*) are scattered throughout the cell. The squamous epithelial dark cells are positioned superior to the light cells. A capillary (*V*) is in proximity to the light cells. Capillaries, however were never observed bordering dark cell. *N* Nucleus; *SV* Scala vestibuli.

not have long processes but are in close contact with contiguous osmiophilic cells and with each other. The osmiophobic cells (Fig. 4) are cuboidal in shape with an irregular spherically shaped nucleus which is located in the middle of the cell. At this power endoplasmic reticulum and mitochondria are seen throughout the cell. The smooth plasma membrane makes contact with adjacent cells and connective tissue. A capillary can be seen in proximity to the osmiophobic cells. *Capillaries* occasionally border the osmiophobic cells, but they were never observed bordering an osmiophilic cell. The squamous (osmiophilic) epithelial cells with their long cytoplasmic processes are visible superior to the osmiophobic cells.

Note in Figs. 5 and 6 the immense size of the nuclear regions of the osmiophilic cells relative to their cytoplasmic processes. The nucleus is irregular in shape and lies very close to the surface of the cell. The cytoplasmic processes in this region infold and create a complex network. The extremities of these infoldings appear to coalesce with the plasma mem-

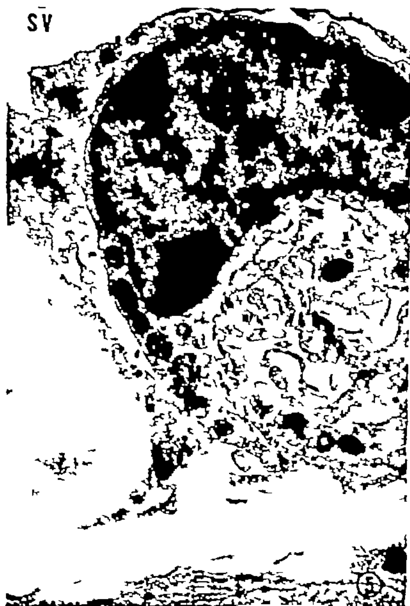


Fig. 5. Electron photomicrograph demonstrating the nuclear region of the osmiophilic cells. The nucleus is irregular and lies close to the surface of the cell. Thin cytoplasmic processes reticulate in the area of the nucleus and expand to enclose mitochondria (M). G Golgi apparatus; N Nucleus; SV Scala vestibuli.

brane to form pinocytotic vacuoles. The cytoplasmic processes which surround the nucleus frequently expand to enclose spherical mitochondria. Golgi apparatus are positioned around the periphery of the nucleus. Interspersed among the mitochondria are free ribosomes and endoplasmic reticulum.

The vasculo-epithelial zone in the cat resembles its counterpart in the guinea pig. The most prominent cytologic characteristic of the epithelial cell is the cytoplasmic processes (Fig. 7 *a*) in the area of the nucleus. These processes form an extensive network which greatly increase the surface area of these cells. The inferior layers of cells contain the same structures seen in the cells of the guinea pig. The electron photomicrograph in Fig. 7 *b* illustrates the organelles which are contained within the cat *epithelial cells*. Numerous *mitochondria*, vesicles and free ribosomes are distributed throughout the cytoplasm. Endoplasmic reticulum are clearly defined. Interesting differences, however were noted between the epithelial cells of the cat and guina pig. In the cat, the epi-

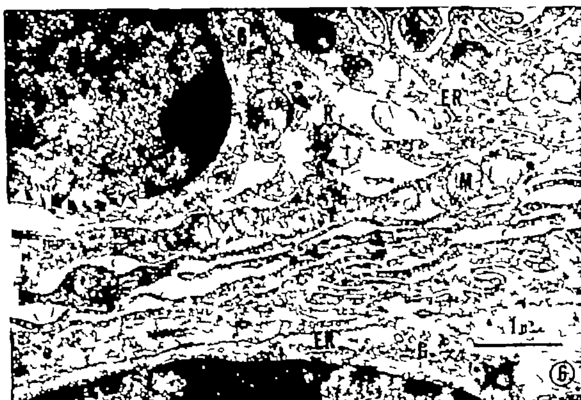


Fig. 6. The cytoplasmic processes of the dark cells infold and create complex network. Frequently Golgi apparata (*G*) are visible close to the nucleus. The distal ends of the cytoplasmic processes appear to coalesce with the plasma membrane to form vacuoles. *ER*, Endoplasmic reticulum, *R* Ribosomes; *M* Mitochondria.

thelial cells are less electron-dense and their nuclei are spherical and do not lie close to the surface of the cell.

DISCUSSION

The vasculo-epithelial zone is a complex of active cells which are in proximity to the medial branches of the spiral artery. The ultrastructure of these cells is indicative of an epithelium which is capable of active transport. The osmophilic epithelial cells of the vasculo-epithelial zone are morphologically similar to the dark cells of the mammalian vestibular system. The vestibular dark cells (Kimura, 1969) which are thought to play a significant role in the active transport of electrolytes, have a dense cytoplasm and consist of numerous plasma membrane processes which are filled with mitochondria. Intracellular inclusions such as endoplasmic reticulum, mitochondria and Golgi apparatus are also exhibited in these cells. These morphological similarities between the epithelial cells of the vasculo-epithelial zone and the vestibular dark cells provides support for the hypothesis that the vasculo-epithelial zone is capable of active transport.

We also noted striking morphologic similarities between the stria vascularis, as described by Smith (1957) Hinojosa & Rodriguez Echandia (1966) and Spoendlin (1967) and the vasculo-epithelial zone. Both have a rich vascular supply and are made up of two types active cell. In both the vasculo-epithelial zone and stria vascularis, dark cells line the surface and are bathed in cochlear fluid (stria vascularis—endolymph vasculo-epithelial zone—perilymph). The light cells lie inferior to the dark

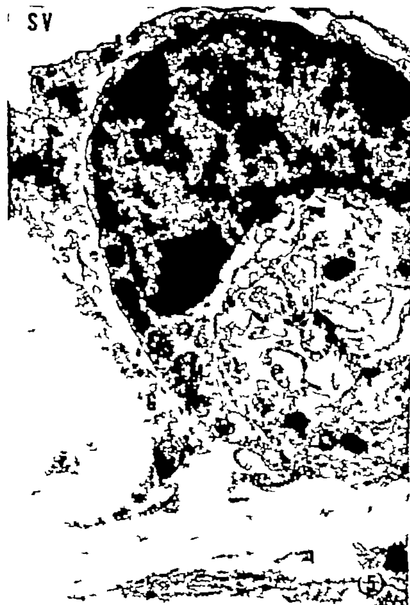
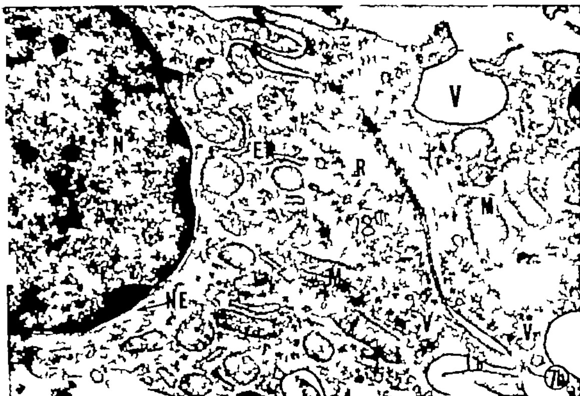


Fig 5 Electron photomicrograph demonstrating the nuclear region of the osmiophilic cells. The nucleus is irregular and lies close to the surface of the cell. Thin cytoplasmic processes reticulate in the area of the nucleus and expand to enclose mitochondria (*M*). *G* Golgi apparatus; *N* Nucleus; *SV* Scala vestibuli.

brane to form pinocytotic vacuoles. The cytoplasmic processes which surround the nucleus frequently expand to enclose spherical mitochondria. Golgi apparatus are positioned around the periphery of the nucleus. Interspersed among the mitochondria are free ribosomes and endoplasmic reticulum.

The vasculo-epithelial zone in the cat resembles its counterpart in the guinea pig. The most prominent cytologic characteristic of the epithelial cell is the cytoplasmic processes (Fig. 7 *a*) in the area of the nucleus. These processes form an extensive network which greatly increase the surface area of these cells. The inferior layers of cells contain the same structures seen in the cells of the guinea pig. The electron photomicrograph in Fig. 7 *b* illustrates the organelles which are contained within the cat epithelial cells. Numerous mitochondria, vesicles and free ribosomes are distributed throughout the cytoplasm. Endoplasmic reticulum are clearly defined. Interesting differences, however were noted between the epithelial cells of the cat and guina pig. In the cat, the epi-



lular inclusions. However several important differences were noted between them. The avian dark cells possess microvilli which protrude into the endolymphatic space and the tegmentum vasculosum and transitional zone are both separated from their underlying capillary network by a basal lamina.

The ultrastructural characteristics of the dark cells of the vasculo-epithelial zone—namely the thin, mitochondria filled cytoplasmic processes, endoplasmic reticulum and Golgi apparatus—suggest an epithelia which may have a secretory function. The inclusions of the light osmiophobic cells, particularly the endoplasmic reticulum, typifies a cellular network which is capable of active transport.

The anatomical relationship between the dark and light cells, their morphology their anatomical similarities to other secreting and absorbing areas and the exposure of these cells to the perilymphatic fluid suggest that the vasculo-epithelial zone plays a role in the maintenance and/or production of perilymph.

ACKNOWLEDGMENT

The authors would like to express their gratitude to Dr E. N Myers for his valuable constructive suggestions.

ZUSAMMENFASSUNG

Die Ultrastruktur der vasculo-epithelial Zone des Spiral-Limbus des Meerschweinchens und der Katze wurde beschrieben. Die vasculo-epithelial Zone besteht aus zwei Zellarten, osmiophilen (dunklen) und osmiophoben (hellen) Zellen. Die osmiophilen Zellen liegen einerseits auf der Oberflache der vasculo-epithelial Zone und andererseits ragen sie in den Perilymph hinein. Diese Zellen besitzen ein dunkles Zytoplasma, welches endoplasmatisches Retikulum, Golgi Apparat und Mitokondrien enthält. Die untere Lage der Zone besteht aus osmiophoben Zellen, welche endoplasmatisches Retikulum und Mitokondrien enthalten. Morphologische Ähnlichkeiten zwischen der vasculo-epithelial Zone und den vestibular dunklen Zellen, der Stria Vascularis und dem Tegmentum Vasculosum wurden beobachtet Die Ultrastruktur der vascular epithelial Zone deutet an, dass diese Zone zu aktivem Transport fähig ist.

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Fig. 7 (a–b). The electron photomicrographs of the vasculo-epithelial zone of the cat illustrate the overall structure (*a*), and intracellular structure (*b*). The cytoplasm contains numerous mitochondria (*M*), vesicles ( ) and vacuoles (*V*) and endoplasmic reticulum (*ER*). A well-developed nuclear envelope (*NE*) is found surrounding the nucleus (*N*). The intricacies of the plasma membrane are evident.

lls and comprise the second layer The capil ry network, in the underlying connective tissue of the vasculo-epithelial zone is not separated from the epithelium by a basal lamina. The same anatomical relationship exists in the stria vascularis. In the stria, cytoplasmic processes extend from the dark cells, past the light cells, to the base of the stria where they surround capillaries. In contrast to the stria, the capillaries in the limbus do not make contact with the osmiophilic (dark) cells. They do, however border the osmiophobic (light) cells. In both the vasculo-epithelial zone and stria vascularis the cytoplasmic processes of the dark cells contain numerous tightly packed mitochondria and the nuclei lie close to the surface of the cell. Histochemical studies (Gerhardt, 1962) have also indicated similarities between the vasculo-epithelial zone and the stria vascularis. Intense DPN D and TPN-D activity were noted in the vasculo-epithelial zone and the stria vascularis in the guinea pig cochlea.

The vasculo-epithelial zone was compared to the inner ear tissue from another species (pigeon) which is known to have secretive and absorptive properties (Dohlman, 1964 1965). The comparison revealed very interesting morphological relationships and similarities between the vasculo-epithelial zone and the tegmentum vasculosum and transitional zone of the avian inner ear The tegmentum vasculosum and transitional zone, which are capable of secretion and absorption, consist of a single layer of alternating dark and light cells (Ishiyama et al., 1969 1970 Ishiyama & Keels, 1970) The avian dark and light cells resemble the dark and light cells of the vasculo-epithelial zone, with respect to overall structure and intracel-

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ÜBER DIE VERTEILUNG DER ACETYLCHOLINESTERASE AKTIVITÄT IM CORTI'SCHEN ORGAN VON FLEDERMÄUSEN

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Abstrakt. Es wurde die Acetylcholinesterase Aktivität in der Cochlea von Microchiroptera nachgewiesen. Damit wurden der Verlauf und die Verteilung der efferenten Fasern dargestellt. Das Ergebnis stimmt im wesentlichen mit den histochemischen und elektronenmikroskopischen Befunden an anderen Säugetierspezies überein. Auffallend war die geringere Zahl efferenter Nervenendungen an den äußeren Haarzellen am Beginn der Basalwindung.

Die Darstellung der Acetylcholinesterase (AChE) Aktivität im Corti'schen Organ wurde von Ishii & Balogh (1968) dazu benutzt, das Verteilungsmuster der efferenten Nervenendigungen an den Haarzellen in der Cochlea der Katze zu studieren. Und zwar sind es nach den neuen *elektronenmikroskopischen Befunden* von Spoendlin (1969) an der Katze die äußeren Haarzellen, an denen die efferenten Nervenfasern enden, während die Mehrzahl der afferenten Nervenendigungen an den inneren Haarzellen liegt. Spoendlin betont die artspezifischen Unterschiede, die im Verlauf und in der Verteilung efferenter und afferenter Fasern in der Cochlea zu finden sind. Um an einer weiteren Säugetiergruppe Kenntnis von der efferenten Innervation der Haarzellen zu erlangen, wurden mit Hilfe der AChE Darstellung Untersuchungen an Fledermausen angestellt. Die besondere Leistungsfähigkeit des akustischen Sinnesorgans der Chiropteren ist ja bekannt (Griffin, 1958)

MATERIAL UND METHODE

Je ein Exemplar von Barbastella barbastellus und Plecotus austriacus (Vespertilionidae) und zwei Exemplare von Rhinolophus hipposideros (Rhinolophidae) wurden in Äthernarkose vom Herzen aus mit Ringerlösung von 37 C und anschließend mit 5%iger Formollösung von 5 C durchspült. Die Formollösung war durch 1/15 molaren Phosphatpuffer auf pH 7 4 eingestellt. Wir ließen die isolierten Schläfenbeine bei 5 C 48 Stunden lang fixieren und entkalkten sie dann 30 Tage in EDTA (Di-Natriumsalz der Äthylendiamintetraessigsäure, 10% (g) ebenfalls bei 5 C. Die Cochleae wurden bei −16 C eingefroren und zu $10\,\mu$ dikken Serienschnitten verarbeitet. Die auf Objektträgern aufgezogenen Schnitte wurden 12 Stunden im Kühlschrank belassen. Zur Darstellung der AChE Aktivität verwendeten wir die Methode von Karnovsky & Roots (1964) Die Schnitte inkubierten wir mit auf den Objektträger getropftem Reagens bei Zimmertemperatur 5 Stunden lang. Die Gegenfärbung erfolgte mit Hämatoxylin. Die Schnitte wurden in Glyzerin eingedeckt. *Zur Kontrolle* der Spezifität der Reaktion wurde auf einzelnen Objektträgern einerseits Physostigminsalicylat (10 molar) zur Hemmung von AChE und Cholinesterase (ChE) zugesetzt, andererseits Silbernitratlösung (10^{-4} molar) zur Hemmung der ChE. Bei einigen anderen Objektträgern wurde als Substrat Butyrylthiocholinjodid anstelle von Acetylthiocholinjodid verwendet. Wieder einige andere Schnitte wurden mit reiner Stammlösung (ohne Substrat) behandelt bei diesen zeigte sich erwartungsgemäß keine Reaktion. Die von uns angewandte Methode

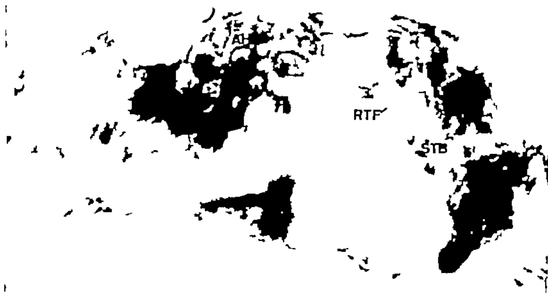


Abb. 1 Darstellung der AChE Aktivität im Corti'schen Organ. *ISB* inneres Spiralbündel *STB* spirales Tunnelbündel *RTF* radiale Tunnelfaser *AHZ* äußere Haarzellen.

nach Karnovsky & Roots hat gegenüber der Koelle schen Methode den Vorteil, keine Metallsulfidniederschläge zur AChE Darstellung zu verwenden, was bei markhältigen Nervenfaserstrecken Fehlergebnisse vermeidet erbst, 1965) Verzichtet haben wir auf den achweis efferenter Fasern nach der von Engström et al. (1966) angegebenen Methode mittels Osmiumtetroxyd-Zinkjodidfärbung, sowie auf die Methode von Hiraide (1970) die auf dem Nachweis alkalischer Phosphatase basiert.

Ishii & Balogh (1968) beurteilten das Ergebnis nach Darstellung der AChE Aktivität an den äußeren Haarzellen mit Hilfe einer vierstufigen Skala (keine Reaktion und drei positive Stufen) Wir verwendeten hingegen, ebenso wie Schuknecht et al. (1959) eine nur dreistufige Skala (negativ schwach positiv stark positiv) dadurch scheint die subjektive Komponente der Beurteilung herabgesetzt. Wir musterten die Schnitte bei 500-facher Vergrößerung durch und beurteilten im Bereiche der Haarzellen insbesondere die Flächenausdehnung des Niederschlages, weniger die Farbintensität. Die Beurteilungen wurden in einem Schema der Cochlea eingetragen.

ERGEBNIS

Nachweise der AChE Aktivität in der Fledermausschnecke zeigten sich an ähnlichen Stellen, wie sie von Schuknecht et al. (1959) von Gacek et al. (1965) und von Balogh & Nomura (1964) an der Katze, von Hilding & Wersäll (1962) am Meerschweinchen, von Ishii et al. (1967) am Totenkopfäffchen und von Nomura & Schuknecht (1965) am Menschen gefunden wurden, nämlich 1) im Verlauf der efferenten Cochlearisfasern, 2) in deren Endigungsbezirken, 3) aber auch an Bildungen, die nichts mit dem efferenten Cochlearissystem zu tun haben, wie z. B an den Ganglienzellen des Ganglion spirale.

Im Stamm des Nervus cochlearis fanden wir einzelne AChE-positive Fasern. Das intraganglionäre Spiralbündel zeigte sich im Vergleich zu den Befunden der oben angeführten



Abb 2 AChE Aktivität im Bereich der äußeren Haarzellen. *EE*, efferente Nervenendigungen, *ASB* äußere Spiralbündel.

Autoren und eigenen Untersuchungen an der Cochlea der Albinoratte kleiner Im Bereich der Lamina spiralis ossea zeigen einzelne Fasern eine deutliche Reaktion. Gelegentlich ist zu erkennen, daß die Fasern nach der *Habenula perforata* in Richtung gegen die Scala vestibuli abbiegen und so das Corti'sche Organ betreten.

Hier schließen sie sich dem inneren Spiralbündel (inner spiral bundle) an, das dadurch immer eine deutliche Tingierung aufwies. Weniger gefärbt, aber noch deutlich erkennbar war das spirale Tunnelbündel (tunnel spiral fibres) Auch die radialen Tunnelfasern (tunnel radial fibres) waren durch Niederschläge von Kupferferrocyanid deutlich sichtbar obwohl sie doch einzeln verlaufen und einen sehr geringen Durchmesser haben, der sich kaum wesentlich von den von Spoendlin (1966) für die Katze angegebenen Maßen von 0,5–1,5 μ unterscheiden dürfte (Abb 1) In Fortsetzung der radialen Tunnelfasern gab es starke Reaktionen im Bereich unmittelbar unterhalb der äußeren Haarzellen. Diese letztgenannten Reaktionen geben Hinweise auf die *Lokalisation der efferenten Synapsen* (Abb 2)

Bei der Bewertung der AChE Aktivität in diesem Synapsenbereich nach unserer dreistufigen Skala (negativ schwach positiv stark positiv) ergab sich folgendes Bild. Schon bei oberflächlicher Musterung der Schnitte war die besondere Intensität der Reaktion in der mittleren Windung eklatant. Ebenso auffällig war der stark positive Befund an der inneren Reihe der äußeren Haarzellen im Vergleich zur äußeren Reihe. Die Beurteilungen wurden zu einer graphischen Darstellung zusammengefaßt (Abb. 3) In Blocks zusammengestellt sind die Befunde der einzelnen Windungen (basale, mittlere und apicale Windung) jeweils für die drei Reihen der äußeren Haarzellen (innere

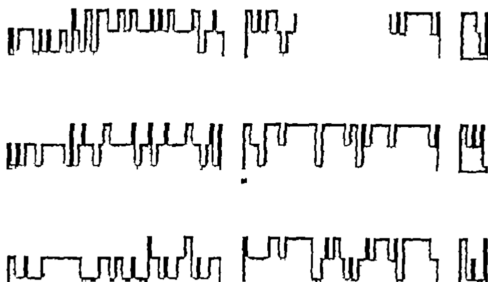


Abb 3 Bewertung der AChE Aktivität im Bereich der efferenten Nervenendigungen an den äußeren Haarzellen. *1* innere Reihe *2* mittlere Reihe; *3* äußere Reihe der äußeren Haarzellen; *B* Basalwindung; *M* Mittelwindung; *A* Apicalwindung. Halbe Höhe der Säulen = schwachpositive Reaktion; ganze Höhe der Säulen = stark positive Reaktion.

und äußere Reihe) Die innere Reihe zeigt im Vergleich zu den beiden anderen Reihen mit Ausnahme des Beginns der Basalwindung die stärksten Reaktionen. Am Beginn der Basalwindung verhalten sich alle drei Reihen untereinander ganz ähnlich. Die Reaktion fehlt oder ist nur schwach positiv Ganz allgemein läßt sich bei allen drei Reihen ein Ansteigen der -itiven Befunde gegen die Mittelwindung mit einem Maximum in der Mitte der Mittelwindung und ein Abfall gegen apical feststellen. Dieser Anstieg beginnt bei der inneren und mittleren Reihe schon in der unteren Hälfte der Basalwindung, bei der äußeren Reihe erst gegen Ende der Basalwindung. Diese Tendenzen zeigten sich bei allen untersuchten Vespertilionidenschnecken Rhinolophus hipposideros konnte für die Auswertung der AChE Aktivität an den Haarzellen nicht verwendet werden, da keine kompletten Schnittserien vorlagen.

Von den Stellen der AChE Aktivität an den Basen der *äußeren Haarzellen abwärts gegen* die Scala tympani ließen sich zwischen den Deiters schen Zellen positive Reaktionen entlang von Nervenfasern feststellen. Wie in der Diskussion der Befunde näher ausgeführt wird, ist die Deutung dieser Nervenfasern schwierig, weil wir entlang der radialen Basilarnervenfasern (basilar nerve fibres) keine Hinweise auf AChE Aktivität fanden.

DISKUSSION DER ERGEBNISSE

Das System efferenter Nervenfasern und -endigungen im Corti schen Organ wurde in den letzten Jahren ausführlich studiert (Zusammenfassungen bei Spoendlin, 1966 Engström et al. 1966 Smith, 1967 und bei Spoendlin, 1968 und Gribenski, 1968) Über den Verlauf der efferenten Fasern ist man durch histochemische, elektronenmikroskopische und experimentell-pathologische Untersuchungen gut orientiert. Im lichtmikroskopischen Bereich erweist sich besonders die Darstellung der AChE Aktivität, die erstmals von Schuknecht et al. (1959) verwendet wurde, als nützlich. Der Beweis für den Zusammenhang zwischen AChE Aktivität und efferenten Fasern gelang diesen selben Autoren sowie Gacek et al. (1965) und Ishii et al. (1967) durch Durchschneidung des olivo-cochlearen Bündels (OCB) da ja die Hauptmasse der efferenten Fasern der Oliva superior entstammt, von wo die Fasern—zum größten Teil gekreuzt—zur Cochlea verlaufen (Rossi & Cortesina, 1965)

Elektronenmikroskopisch wurden an den äußeren Haarzellen zwei Typen von Nervenendigungen nachgewiesen, die sich voneinander am auffallendsten durch die Zahl der Vesikeln unterscheiden. Nach Durchtrennung des OCB konnte festgestellt werden, daß die mit reichlich Vesikeln versehenen Endigungen zugrunde gingen (Iurato 1962 Smith & Rasmussen, 1965) und sie daher als die efferenten Endigungen aufzufassen sind. Kaneko & Daly (1968) konnten elektronenmikroskopisch mit Hilfe der Karnovsky-Methode die AChE an der Plasmamembran der radialen Tunnelfasern und deren vesikelreichen Nervenendigungen lokalisieren, nicht aber in diesen Vesikeln, ein Befund, der mit den elektrophysiologischen Untersuchungen von Tanaka & Katsuki (1966) übereinstimmt, die eine von Acetylcholin verschiedene Transmittersubstanz annehmen.

Es erscheint notwendig, unsere Befunde von der Fledermauscochlea mit denen der bisher untersuchten Arten sowohl bezüglich des Nervenfaserverlaufs als auch bezüglich der Nervenendigungen zu vergleichen.

Nach den Untersuchungen von Spoendlin, 1969 an der Katze sind afferente und efferente Nervenfasern in ihrem Verlauf im Corti'schen Organ getrennt. Efferente Fasern verlaufen im inneren Spiralbündel und im Tunnelspiralbündel, kreuzen als radiale Tunnelfasern den inneren Tunnel und enden an den äußeren Haarzellen. Die von den äußeren Haarzellen her kommenden afferenten Fasern, die an Zahl geringer sind als die von den inneren Haarzellen her kommenden, verlaufen als äußere Spiralbündel und als radiale Basilarnervenfasern zur Habenula perforata.

Beim Chinchilla ist der Verlauf nach Smith (1967) ähnlich, nur ist die Aufteilung der radialen Fasern im Bereich des inneren Tunnels in efferente und afferente Bündel nicht gegeben, d. h. sowohl die radialen Tunnelfasern wie die radialen Basilarnervenfasern sind teils afferent teils efferent. Bei einem anderen Nager dem Meerschweinchen, gibt es ebenfalls keine klare Trennung der afferenten und efferenten, den Tunnel kreuzenden Fasern (Spoendlin, 1969 S. 254) doch kreuzt der größte Teil der efferenten Fasern den Tunnel als radiale Tunnelfasern (Kaneko & Daly 1968)

Unsere Befunde an der Chiropterencochlea zeigen, daß bei diesen Arten keine klare topographische Gliederung in efferente und afferente Faserbündel vorkommt. Die Hauptmasse der efferenten Fasern scheint als radiale Tunnelfasern zu den äußeren Haarzellen zu ziehen. Es fanden sich jedoch auch Niederschläge entlang von Nervenfasern zwischen den Deitersschen Zellen unterhalb der äußeren Haarzellen. Diese Niederschläge ließen sich bis an die Basilarmembran verfolgen, wo sie in Richtung Modiolus umbogen. Da aber in Fortsetzung dieser Fasern keine Niederschläge im Bereich der radialen Basilarnervenfasern nachgewiesen werden konnten, muß die Deutung dieser Strukturen offen bleiben. Eine mögliche Erklärung wäre die Deutung als Summierung von Querschnitten einzelner spiralig verlaufender efferenter Fasern in den äußeren Spiralbündeln, wie sie von Kaneko & Daly (1968) zwischen den AChE negativen afferenten Fasern nachgewiesen werden konnten. Der optische Eindruck suggeriert aber eher daß es sich um Fasern handelt, die aus dem Gebiet der Basilarnervenfasern aufsteigen.

Die Endigungen der efferenten Nervenfasern liegen einerseits an den äußeren Haarzellen, andererseits an den afferenten Dendriten im Bereich der inneren Haarzellen (Smith, 1967) und zwar stammen nach Fex (1968) die Endigungen an den äußeren Haarzellen von den gekreuzten Fasern des OCB die an den inneren Haarzellen von den ungekreuzten Fasern. Über die Endigungen der efferenten Fasern an den afferenten Dendriten unterhalb der inneren Haarzellen lassen sich an unseren Präparaten leider keine Feststellungen treffen da die positive Reaktion des inneren Spiralbündels keine feinere Beurteilung zuläßt. Hingegen sind die Niederschläge an den Synapsen äußeren Haarzellen einer lichtmikroskopi Untersuchung zugänglich. Ishii & (1968) fanden bei der Katze im

Basalwindung eine nur schwache efferente Innervation, die sehr bald rasch zunahm, um erst in der Apikalwindung allmählich abzusinken. Diese Verhältnisse zeigten sich in allen drei Reihen der *äußeren Haarzellen*, die apicale Abnahme war am deutlichsten bei der äußeren Reihe. In unseren Präparaten war im Prinzip dasselbe Muster zu finden. Allerdings war der basale Bereich geringer efferenter Innervation weiter ausgedehnt, nämlich bis ungefähr bis zur Mitte der Basalwindung, und das Maximum der efferenten Innervation lag in der mittleren Windung. Im Gegensatz zu der der Katze und den Vespertilionidae gemeinsamen Armut der efferenten Innervation am Beginn der Basalwindung, stehen die Befunde von Hilding & Wersäll (1962) am Meerschweinchen, bei dem sich ein Maximum der efferenten *Innervation in der Basalwindung* und eine *successive Abnahme nach apical fand* (lichtmikroskopische Bewertung der AChE Aktivität) sowie der elektronenmikroskopischen Untersuchungen von Smith & Sjöstrand (1961), die ebenfalls beim Meerschweinchen zu demselben Ergebnis kamen, allerdings nach Auswertung nur weniger Haarzellen und ihrer Synapsen.

Es erhebt sich nun die Frage, inwieweit morphologischen Befunde ihr Korrelat im funktionellen Verhalten finden.

Die Funktion des efferenten Systems wird in verschiedenen Abschnitten des Sammelbandes „Hearing mechanism in vertebrates" ausführlich diskutiert und als feedback System gedeutet. Kurz zusammengefaßt läßt sich sagen, daß bei elektrischer Stimulierung des OCB eine Erhöhung der Mikrophonpotentiale und eine Erniedrigung der Aktionspotentiale des N. cochlearis auftreten. Bei der Katze konnten Kittrell & Dalland (1969) nachweisen, daß eine Reizung des OCB bei gleichzeitiger akustischer Stimulation nur im Bereiche zwischen 1 kHz und 13 kHz zu einer Erhöhung der Mikrophonpotentiale führte. Die Tatsache, daß bei Frequenzen unter 1 kHz und über 13 kHz kein Stimulationseffekt auftritt, steht in guter Übereinstimmung mit der fast mangelnden efferenten Innervation im apicalsten und basalsten Bereich, wie auch schon Ishii und Balogh nach Vergleichen mit den Versuchen von Wiederhold (1967) betonten. Praktisch wichtige Effekte der Efferenten, zumindest des gekreuzten OCB, sind Verbesserung der Frequenzdiskrimination (Klinke et al., 1969) und Maskierung störender Geräusche (Dewson III, 1968). Keidel (1970) schreibt von einer Wirkungsgradverstellung aller synaptischen Übergänge durch rückläufige Faserzüge. Das OCB ist ein Teil dieser Bahnen zur „optimalisierenden akustischen Informationsselektion.

Es ist überraschend, daß in der Basalwindung der untersuchten Fledermäuse die efferente Innervation relativ spärlich ist. Man müßte erwarten, daß für die Analyse der Ultraschallfrequenzen in der Basalwindung eine gute Frequenzdiskrimination und geringe Störanfälligkeit *notwendig sind. Abgesehen davon, daß* für die Frequenzdiskrimination auch zentrale Mechanismen eine Rolle spielen (Neuweiler, 1970) und daß bei der Echopeilung vor allem Zeitdifferenzen zur Entfernungsschätzung benützt werden (Simmons, 1970), muß man aber bedenken, daß diffuse Störgeräusche im Ultraschallbereich in der natürlichen Umwelt der Fledermäuse nicht vorkommen. Peillaute von Artgenossen ergeben gerichtete Schallfelder und sind daher leichter zu identifizieren. Wenn man Fledermäuse durch ungerichtete Ultraschallgeräusche stört, so wirkt sich die Störung *in den höheren Frequenzbereichen (über 50* kHz) deutlich aus. Plecotus townsendii hatte mehr Mißerfolge beim Erkennen von Hindernissen, wenn er durch Störgeräusche mit Frequenzen über seiner Peillautfrequenz (25–45 kHz) beschallt wurde. Im Bereich seiner Peillautfrequenz wurde er viel weniger beeinträchtigt (Griffin et al., 1963). Obwohl über die mechanische Frequenzanalyse in der Fledermauscochlea nichts bekannt ist, kann man doch annehmen, daß Frequenzen wie 25–45 kHz erst in der oberen Basalwindung zur Wirkung kommen. Die Dimensionen der Basilarmembran der Chiropteren sind an den Frequenzbereich angepaßt und für die Analyse

hoher Frequenzen besonders geeignet (Hinchcliffe & Pye, 1968) Zumindest bei Plecotus, den wir untersuchten, gibt es in der oberen Basalwindung schon mehr efferente Nervenendigungen und damit eine Erklärung für die geringere Störanfälligkeit bei Frequenzen unter 50 kHz.

SUMMARY

The acetylcholinesterase activity in the cochlea of bats (Microchiroptera) was demonstrated. With this method the course and the distribution of the efferent fibres are shown. The result is in greement with histochemical and electron microscopic data from other species of mammals. The only remarkable difference to other species is the lower number of efferent nerve fibres ending on the outer hair cells in the first half of the basal turn.

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Basalwindung eine nur schwache efferente Innervation, die sehr bald rasch zunahm, um erst in der Apikalwindung allmählich abzusinken. Diese Verhältnisse zeigten sich in allen drei Reihen der äußeren Haarzellen, die apicale Abnahme war am deutlichsten bei der äußeren Reihe In unseren Präparaten war im Prinzip dasselbe Muster zu finden. Allerdings war der basale Bereich geringer efferenter Innervation weiter ausgedehnt, nämlich bis ungefähr bis zur Mitte der Basalwindung, und das Maximum der efferenten Innervation lag in der mittleren Windung. Im Gegensatz zu der der Katze und den Vespertilionidae gemeinsamen Armut der efferenten Innervation am Beginn der Basalwindung, stehen die Befunde von Hilding & Wersäll (1962) am Meerschweinchen, bei dem sich ein Maximum der efferenten Innervation in der Basalwindung und eine successive Abnahme nach apical fand (licht mikroskopische Bewertung der AChE Aktivität) sowie der elektronenmikroskopischen Untersuchungen von Smith & Sjöstrand (1961) die ebenfalls beim Meerschweinchen zu demselben Ergebnis kamen, allerdings nach Auswertung nur weniger Haarzellen und ihrer Synapsen.

Es erhebt sich nun die Frage, inwieweit diese morphologischen Befunde ihr Korrelat im funktionellen Verhalten finden.

Die Funktion des efferenten Systems wird in verschiedenen Abschnitten des Sammelbandes „*Hearing mechanism in vertebrates*" ausführlich diskutiert und als feedback System gedeutet. Kurz zusammengefaßt läßt sich sagen, daß bei elektrischer Stimulierung des OCB eine Erhöhung der Mikrophonpotentiale und eine Erniedrigung der Aktionspotentiale des N cochlearis auftreten. Bei der Katze konnten Kittrell & Dalland (1969) nachweisen, daß eine Reizung des OCB bei gleichzeitiger akustischer Stimulation nur im Bereiche zwischen 1 kHz und 13 kHz zu einer Erhöhung der Mikrophonpotentiale führte. Die Tatsache, daß bei Frequenzen unter 1 kHz und über 13 kHz kein Stimulationseffekt auftritt, steht in guter Übereinstimmung mit der fast mangelnden efferenten Innervation im apicalsten und basalsten Bereich, wie auch schon Ishii und Balogh nach Vergleichen mit den Versuchen von Wiederhold (1967) betonten. Praktisch wichtige Effekte der Efferenten, zumindest des gekreuzten OCB sind Verbesserung der Frequenzdiskrimination (Klinke et al. 1969) und Maskierung störender Geräusche (Dewson III 1968) Keidel (1970) schreibt von einer Wirkungsgradverstellung aller synaptischen Übergänge durch rückläufige Faserzüge. Das OCB ist ein Teil dieser Bahnen zur „optimalisierenden akustischen Informationsselektion

Es ist überraschend, daß in der Basalwindung der untersuchten Fledermäuse die efferente Innervation relativ spärlich ist. Man müßte erwarten, daß für die Analyse der Ultraschallfrequenzen in der Basalwindung eine gute Frequenzdiskrimination und geringe Störanfälligkeit notwendig sind. Abgesehen davon, daß für die Frequenzdiskrimination auch zentrale Mechanismen eine Rolle spielen (Neuweiler 1970) und daß bei der Echopeilung vor allem Zeitdifferenzen zur Entfernungsschätzung benützt werden (Simmons, 1970) muß man aber bedenken, daß diffuse Störgeräusche im Ultraschallbereich in der natürlichen Umwelt der Fledermäuse nicht vorkommen. Peillaute von Artgenossen ergeben gerichtete Schallfelder und sind daher leichter zu identifizieren. Wenn man Fledermäuse durch ungerichtete Ultraschallgeräusche stört, so wirkt sich die Störung *in den höheren Frequenzbereichen* (über 50 kHz) deutlich aus. Plecotus townsendi hatte mehr Mißerfolge beim Erkennen von Hindernissen, wenn er durch Störgeräusche mit Frequenzen über seiner Peillautfrequenz (25–45 kHz) beschallt wurde Im Bereich seiner Peillautfrequenz wurde er viel weniger beeinträchtigt (Griffin et al., 1963) Obwohl über die mechanische Frequenzanalyse in der Fledermauscochlea nichts bekannt ist, kann man doch annehmen, daß Frequenzen wie 25–45 kHz erst in der oberen Basalwindung zur Wirkung kommen. Die Dimensionen der Basilarmembran der Chiropteren sind an den Frequenzbereich angepaßt und für die Analyse

erythrocyte hemolysate showed that with the reaction from malate to oxalacetate, MDH bands were not visualized at pH 7.8–7.9 At pH 8.5 bands were visualized and were still stronger at pH 9.0–9.2. In post-mortem sera, some samples showed MDH bands at pH 7.8–7.9 and there was continuous increase with incubation at pH 8.5 and at pH 9.0–9.2. Quantitative estimates of the total MDH for normal serum showed a trace at pH 7.4 at pH 8.5 values ranged between 15 and 20 mIU/ml and at pH 10 between 17 and 22 mIU/ml. For fresh erythrocyte hemolysate the values were 10 mIU/ml, 180 mIU/ml and 465 mIU/ml, respectively Shortening of the incubation time from 2 h to 25 min, made the bands weaker in all test fluids. Increasing the time for the electrophoretic run from 25 min to 50 min had no effect.

The comparative quantitative MDH analyses in mIU/ml were performed using samples of perilymph of 8 post-mortem ears together with the corresponding CSF and serum samples. In perilymph the Boehringer and Sohn standard clinical method gave an average MDH value of 1 100 (range 750–1 459) mIU/ml while with the method of King an average figure of 900 (range 600–1 025) mIU/ml was obtained. The reaction in opposite order from malate to oxalacetate, at pH 10 (Thorne et al., 1963) yielded an average value of 42 (range 28–53) mIU/ml. Quantitative analyses of lactate dehydrogenase (LDH) using the standard clinical method of Boehringer gave an average of 1 800 (range 768–3 537) mIU/ml in these samples.

Comparative data on serum and CSF showed MDH activities, by the Boehringer and Sohn and the King methods, that were generally much lower than in perilymph, of the order of 100–200 mIU ml. However one serum and one CSF sample showed very high activity and two serum samples total inhibition. In the malate–oxalacetate reaction at pH 10 (Thorne et al., 1963) the values for serum were substantially higher (average 180, range 60–350 mIU/ml) than those for perilymph and CSF which were of similar order In two CSF samples, however no values were recorded because a reaction in the opposite direction developed. For LDH, greatly varying data were recorded from these fluids, ranging from complete inhibition in one sample to the maximum value, 3 385 mIU/ml. In addition, one CSF sample showed very high activity beyond the range possible to measure with the present test arrangement.

The possibility that an endogenous substrate effects the results was tested at pH 8.5 (Katz & Kalow 1965), with no sodium L-malate added. Fig. 1 shows the results in five post mortem sera, the mobility of the bands 1–3 corresponds to those of LDH_{1-3}. Bands 1 and 2 represent the activity areas of MDH_3 and MDH also. The application point corresponds to that of LDH which is the same as MDH_1. In one serum, a weak band of LDH mobility was found. One sample failed to show any bands.

COMMENTS

In the quantitative analyses using the reaction order from oxalacetate to malate the perilymph values with the Boehringer and Sohn meth[illegible] were about 200 mIU/ml larger than t[illegible] tained with the King method Th[illegible] excepted since the latter employ[illegible] pyruvate from the sample. Ser[illegible] values showed an unreasonably [illegible] some being abnormally low or show[illegible] [illegible] inhibition, and a few were very high Only with the spectrophotometric method of Thorne et al at pH 10 in the order from malate to oxalacetate were stable data obtained.

The presence of an endogenous substrate in the areas of LDH_{1-4} (corresponding t[illegible] MDH_{3-4}) is one proof of the fact that in MDH analyses, in the reaction order from malat[illegible] to oxalacetate, inclusion of endogenous [illegible] possible Consequently in the direction from oxalacetate to malate, the pyruvate [illegible] the sample is converted into lactate [illegible] minations of MDH_{3-4} and MDH_4 [illegible] reflect those of LDH_{1-3} and LDH [illegible]

[illegible]

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MALATE DEHYDROGENASE IN POST MORTEM PERILYMPH

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Abstract. Factors affecting the determination of malate dehydrogenase (MDH) activity in post-mortem serum, cerebrospinal fluid (CSF) and perilymph were studied using reactions from malate⇌oxalacetate in both directions. The former reaction order gave stable data which were much lower than those obtained with the reverse order this was thought to be mainly due to different enzyme kinetics. The activity in serum was about double that in CSF and perilymph in malate→oxalacetate reaction. In reverse reaction perilymph MDH values were generally much higher than those of serum or CSF Since these fluids contain several enzymes utilizing the same coenzymes only data obtained in exactly same experimental conditions can be compared with each other

In a recent paper we (Palva et al 1970) discussed malate dehydrogenase (MDH) isoenzyme pattern in post mortem perilymph and dolymph. This pattern was similar in cerebronal fluid (CSF) and perilymph, but in serum differed from both. In the former fluids, MDH_1 dominated strongly while the slow-moving and fast moving isoenzymes showed much lower percentages. The total perilymph MDH values, based on electrophoretic areal measurements and related to CSF and serum values, were considerably lower than reported hitherto (Silverstein & Schuknecht, 1966 Silverstein 1966)

In a search for a cause for these differences, the technical factors employed in the demonstration of MDH were thought to play a major role. Therefore, further studies of MDH using different technical procedures were carried out in an attempt to obtain data serving to clarify the factors that affect the quantitative MDH measurements.

METHOD AND MATERIALS

The basic method consisted in dehydrogenation from malate to oxalacetate at pH 8.5 as used by Katz & Kalow (1965) Performed in this order at or about physiological pH 7 4 small amounts of oxalacetate may inhibit the oxidation of malate. Therefore to obtain comparative data on the effect of pH, the bands were studied also at pH 7 8–7 9 and pH 9 0–9.2. The electrophoretic run itself was made according to the direction of Wieme using a field strength of 25 V/cm for 25 min in agarose gel, at pH 8 6 2 µl of post-mortem serum was generally employed as the test fluid, diluted 1 2 Other test fluids consisted of normal serum, fresh erythrocyte hemolysate Boehringer and Sohn pig heart MDH preparation, and post-mortem perilymph. Incubation time was generally 2 h, but 25 min was used in one series to obtain comparative data. In electrophoresis also 25 min and 50 min runs were compared. For quantitative analyses of post mortem serum, CSF and perilymph, the reactions from malate to oxalacetate (Katz & Kalow 1965) after electrophoresis and from oxalacetate to malate directly from the sample (Boehringer and Sohn King, 1965) were used. In addition, tests in the former direction straight from the sample were made at the optimum pH 10 (Thorne et al., 1963).

RESULTS

Analyses on several normal sera, Boehringer and Sohn pig heart MDH preparation and fresh

test fluids containing several enzymes and utilizing the same coenzymes is a very difficult task. Even if one attempts to measure only one reaction, it is possible that, because of the presence of different enzymes and endogenous substrates, oxidative and reductive processes occur at the same time over which the examiner has no control. This may result in total inhibition, reverse reactions or very high or low values, all of which were observed in our series. Great caution should therefore be excercised in evaluating various data. The results are comparable only if performed in the same manner technically. Moreover there is the fundamental difference between the direction from oxalacetate to malate (Boehringer and Sohn, King), which favours the soluble enzyme and the order from malate to oxalacetate (Thorne et al., 1963), which favours the mitochondrial enzyme and in which the soluble enzyme is inhibited by too large concentrations of substrate. Thus also the quantitative values obtained with these two methods are totally different, the results can be compared with each other only when the same reaction order and a technically identical procedure is used.

ZUSAMMENFASSUNG

Malatdehydrogenase Aktivität in post mortem Serum, Zerebrospinalflüssigkeit und Perilymphe wurde in beiden Reaktionsrichtungen, Malat ⇄ Oxalacetat, unter verschiedenen Bedingungen untersucht. Die Aktivitätswerte in Richtung Malat → Oxalacetat waren stabil, aber viel kleiner als in entgegengesetzter Richtung, was wahrscheinlich auf die Enzymkinetik zurückzuführen ist. Die Serumaktivität war mit Perilymphe und Liquor in Richtung Malat → Oxalacetat mehr als doppelt so gross, aber noch viel grösser zugunsten der Perilymphe in entgegengesetzter Richtung. Wenn die Testflüssigkeiten viele Enzyme enthalten, die dieselben Coenzyme gebrauchen, können die Aktivitätswerte nur mit den in genau identischen Verhältnissen gewonnenen Werten verglichen werden.

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DEVELOPMENT OF MUCOUS GLANDS IN THE HUMAN EUSTACHIAN TUBE

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Abstract The object of the present study was to elucidate the development of the mucous glands in the Eustachian tube. 47 tubes from 33 foetuses aged 11 to 27 menstrual weeks were studied by the PAS, PAS-Alcian blue, and osmium whole mount methods. All tubes were freed and stained in toto, and all glands were examined and counted. Gland formation was found to start in the 12th menstrual week in the lateral wall of the rhinopharynx, caudally to the tubal orifice, and to spread towards the tube. At the beginning of the 13th menstrual week gland formation started in the tube, first in the medial, and 10 days later in the lateral wall. The glands were laid down first in the pharyngeal orifice and gradually spread into the tympanic direction, reaching the middle of the tube in the course of 14 days. Up t the 17th menstrual week about 7 new glands were laid down weekly from the 17th to the 20th week approx. 9 During the subsequent weeks the intensity of gland gradually decreased, and after the 26th there is probably no further gland formation. the 27th week the tube contained an average of 86 glands, 50 in the pharyngeal quarter and 36 in the second quarter No glands were present in the tympanic half. In the medial wall there was an average of 46 glands, in the lateral wall 40. By means of quantitative studies the development and growth of the individual glands are elucidated.

That seromucous glands are present in the wall of the Eustachian tube is well-known, but knowledge concerning their embryology anatomy physiology and pathology is still very inadequate.

The development of the tubal glands has not been described previously Only *Eggston & Wolff* (1947) mentioned glands in the medial wall of the tube in a 5-mounth foetus. Students of the tube around birth have mentioned only that it contains numerous seromucous glands, without entering into details of their anatomy

Those who have studied the anatomy and histology of the tube in children and adults state that the glands extend from the pharyngeal orifice to the middle of the tube (*Moos*, 1874 *Politzer* 1908 *Zöllner* 1942) *Graves & Edwards* (1944) found the glandular layer to vary individually and according to age. It is most pronounced in the pharyngeal orifice and in the medial wall.

The object of the present study was to show when, where, and how glandular development in the tube starts and how it continues through foetal development. To solve these problems the author chose whole-mount methods, dissecting the entire tube free, staining it in toto examining and counting all glands

MATERIAL AND METHODS

The material comprises 47 tubes from 33 foetuses from the 11th to the 27th menstrual week (age distribution shown in Fig. 8) 16 were males and 17 females To determine age the crown–rump length (CR length) and foot length were measured. Foetal age is stated in menstrual weeks, calculated from Streeter's tables (*Streeter* 1921) on the basis of the CR length.

The foetuses were fixed in formol alcohol (2 parts absolute alcohol and 1 part concentrated

neutral formalin) or in Lillie s neutral formalin fixative (Barka & Anderson, 1963) By a sagittal section through the middle of the nose rhinopharynx, and pharynx the foetal head was divided into a right and a left side To be able to evaluate where the glands develop first and how their spread takes place the mucous membrane from the entire nose, rhinopharynx, pharynx, and hypopharynx was freed en bloc (Figs. 1 2) The pharyngeal orifice of the tube was located and the tube was bougied with a soft rubber bougie 1/4–1/2 mm thick. Thereafter the tympanic membrane and the entire middle-ear cavity including the ossicles, were dissected free through the temporal region. The tip of the bougie in the middle ear was visible through the tympanic membrane, and thereafter the course of the tube could be determined. Preparation of the tube was performed from without, both from the pharyngeal and from the tympanic end. The still cartilaginous pyramid and cochlea were removed, preserving the middle-ear and tubal mucosa in toto At the pharyngeal end all the tissue around the tube as well as the pterygoid process with the tensor veli palatini muscle were removed, leaving the tube intact (Fig. 3) To obtain uniform penetration of the dye through the tubal wall and to be able to study the tubal mucosa, the tubes of most foetuses were cut along the roof thus demarcating the medial and lateral walls (Figs. 1 2, 4 5) The cut prolonged around the entire middle ear which was thereby opened.

Three-whole-mount methods were used. Their principle is staining of whole, uncut specimens followed by clearing of the superfluous background staining, making the preparations more or less transparent. These methods have previously been used by the author in studies of the tracheal and bronchial glands in which the procedure, advantages, and disadvantages of whole-mount staining of tissue of different thickness were described. (1) PAS staining (Moe, 1952 Tos, 1966) in which goblet cells and glands stain red on a transparent background (Figs. 1 3 4) (2) PAS-Alcian blue staining (Tos, 1970 *a*) in which goblet cells and glands stain bluish-purple on a pale blue background (Figs. 3 5) (3) Osmium tetroxide staining (Tos, 1966) in which only the glands stain black on a pale brown background (Fig. 2) After the staining the preparations were stored in anise oil and studied in the stereomicroscope at a magnification of × 40 The length of the tube, its inner circumference at the pharyngeal and tympanic orifices as well as in the middle ear were measured. The tube was divided into four equally long quarters, the first (pharyngeal) second, third, and fourth (tympanic) quarter (Figs. 9 10) In all 47 tubes all glands were counted. Moreover separate gland counts were made in each quarter and in the medial and lateral wall separately

RESULTS

Structure and Growth of Glands

By studying the glands in sections and whole mounts from foetuses of different age groups it was possible to follow the development of each individual gland in the tube. The structure and growth of the gland proved to be the same in the tube as in the trachea (Tos, 1966) and main bronchi (Tos, 1968 *a*) and will therefore only be briefly summarized (Fig. 6) Each tubal gland starts its development intra-epithelially by division of the basal cells. During further cell division the glandular primordium grows down into the lamina propria, forming a solid cylinder 100–150 μ long and 20–35 μ in diameter (developmental stage 1) (Fig. 3) A few cells of the glandular primordium start forming mucin, secreted into the intercellular spaces. In the middle of the solid cylinder there forms, in this way a mucin-filled pond" which enlarges to make a canal debouching into the tubal lumen (developmental stage 2) The canal of the glandular primordium later becomes the main excretory duct of the gland The glandular primordium undergoes dichotomous division into two lateral ducts which also form lumina (developmental stage 3) Each of the lateral ducts again undergoes dichotomous division and acquires a lumen.

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The foetuses were fixed in formol alcohol (2 parts absolute alcohol and 1 part concentrated

neutral formalin) or in Lillie's neutral formalin fixative (Barka & Anderson, 1963) By a sagittal section through the middle of the nose rhinopharynx, and pharynx the foetal head was divided into a right and a left side To be able to evaluate where the glands develop first and how their spread takes place, the mucous membrane from the entire nose, rhinopharynx, pharynx, and hypopharynx was freed en bloc (Figs. 1 2) The pharyngeal orifice of the tube was located and the tube was bougied with a soft rubber bougie 1/4–1/2 mm thick. Thereafter the tympanic membrane and the entire middle-ear cavity including the ossicles, were dissected free through the temporal region. The tip of the bougie in the middle ear was visible through the tympanic membrane and thereafter the course of the tube could be determined Preparation of the tube was performed from without, both from the pharyngeal and from the tympanic end. The still cartilaginous pyramid and cochlea were removed, preserving the middle-ear and tubal mucosa in toto At the pharyngeal end all the tissue around the tube as well as the pterygoid process with the tensor veli palatini muscle were removed, leaving the tube intact (Fig. 3) To obtain uniform penetration of the dye through the tubal wall and to be able to study the tubal mucosa, the tubes of most foetuses were cut along the roof thus demarcating the medial and lateral walls (Figs 1 2, 4 5) The cut prolonged around the entire middle ear which was thereby opened.

Three-whole-mount methods were used. Their principle is staining of whole, uncut specimens followed by clearing of the superfluous background staining, making the preparations more or less transparent. These methods have previously been used by the author in studies of the tracheal and bronchial glands in which the procedure, advantages, and disadvantages of whole-mount staining of tissue of different thickness were described. (1) PAS staining (Moe, 1952 Tos, 1966) in which goblet cells and glands stain red on a transparent background (Figs. 1 3 4) (2) PAS-Alcian blue staining (Tos, 1970 *a*) in which goblet cells and glands stain bluish-purple on a pale blue background (Figs. 3 5) (3) Osmium tetroxide staining (Tos, 1966) in which only the glands stain black on a pale brown background (Fig. 2) After the staining the preparations were stored in anise oil and studied in the stereomicroscope at a magnification of ×40 The length of the tube, its inner circumference at the pharyngeal and tympanic orifices as well as in the middle ear were measured. The tube was divided into four equally long quarters, the first (pharyngeal), second, third, and fourth (tympanic) quarter (Figs. 9 10) In all 47 tubes all glands were counted. Moreover separate gland counts were made in each quarter and in the medial and lateral wall separately

RESULTS

Structure and Growth of Glands

By studying the glands in sections and whole mounts from foetuses of different age groups it was possible to follow the development of each individual gland in the tube The structure and growth of the gland proved to be the same in the tube as in the trachea (Tos, 1966) and main bronchi (Tos, 1968 *a*) and will therefore only be briefly summarized (Fig. 6) Each tubal gland starts its development intra-epithelially by division of the basal cells. During further cell division the glandular primordium grows down into the lamina propria, forming a solid cylinder 100–150 μ long and 20–35 μ in diameter (developmental stage 1) (Fig. 3) A few cells of the glandular primordium start forming mucin, secreted into the intercellular spaces. In the middle of the solid cylinder there forms, in this way a mucin-filled "pond" which enlarges to make a canal debouching into the tubal lumen (developmental stage 2) The canal of the glandular primordium later becomes the main excretory duct of the gland The glandular primordium undergoes dichotomous division into two lateral ducts which also form lumina (developmental stage 3) Each of the lateral ducts again undergoes dichotomous division and acquires a lumen









Fig 1 Freed mucosa from the tube, rhinopharynx, pharynx, and hypopharynx and nose (*N*) including the inferior and medial turbinate. In the lateral part of the rhinopharynx, below the tubal orifice, groups of goblet cells and glandular primordia are visible as small dark dots (*arrows*). There are no glands or goblet cells in the rhinopharynx cranially to the tubal orifice or in the posterior part of the nose. The tube has been cut along the roof, and in the medial (internal) wall (*I*), close to the pharyngeal orifice there are couple of glandular primordia. In the palatine mucosa (*P*) also a few glandular primordia. Outlines of the uvula (*U*), tongue (*T*), epiglottis (*E*), and larynx (*L*) as well as drum (*D*) with the malleus are visible. PAS-stained whole mount from a foetus aged 13 menstrual weeks. ×10 (CR length 69 mm).

Fig 2 Mucous membrane from the tube, rhinopharynx (*R*), pharynx (*P*), and nose (*N*) from a 14-week foetus (CR length 74 mm). In the rhinopharynx and upper part of the pharynx many glandular primordia. The tube has been cut in the upper part of the medial wall, where a few glandular primordia (*arrow*) are seen in the pharyngeal part. No glands in the posterior part of the nose and at the anterior lip f the pharyngeal orifice (*O*), while glands are laid down at the posterior lip. The tensor tympani muscle (*T*) is well-developed. Below the tube the levator veli palatini muscle passes towards the soft palat (*S*). In the posterior part of the hard palate (*H*) glandular primordia are visible. Osmium-tetroxide-stained whole mounth. ×9



Fig. 5 Tube with drum and middle-ear mucosa (*M*) from ?7-week foetus (CR length 230 mm). The tube has been cut open and smoothed out. I the lateral wall of layers of glands which have spread to the middle of the tube. The glandular layer from the medial wall (*I*) has been removed, and only the glandular orifices are visible as small, dark dots. Along the rhinopharynx there are groups of goblet cells. Blood vessels are visible in the tympanic part of the tube and in the drum. PAS-Alcian blue-stained whole mount. × 5

At the same time, the quantity of mucin in the cells of the main duct increases (developmental stage 4) This gives rise to four ducts which again undergo dischotomous division making eight new buds which become tubules. In the ampulla of the main duct, there forms stratified ciliated columnar epithelium with goblet cells, and in the tubular cells the quantity of mucin increases (developmental stage 5) During the first stages the glandular development is regular but during their continued growth it is dependent upon the space available for further development. The division of the buds is still dichotomous, but only some of the buds develop further at each division. From each new pair of buds either both or only one will develop further or else both remain at the same developmental stage. However the buds which do not develop further preserve their ability for division and may continue their development later

Already at the time of formation of the main-duct lumen, the cellular differentiation starts, mucinous granules forming inside the cells. After the 5th developmental stage the entire duct system is highly PAS positive (Fig. 4) except at the bud ends where there are several layers of undifferentiated, PAS-negative

Fig. 3 The tube with parts of the rhinopharynx from 15-week foetus (CR length 95 mm) The tube is intact. Below the tube the levator veli palatini muscle (*L*) is seen. In the medial wall several glands in developmental stages 1 and 2 (*arrows*). The glands have reached the middle of the tube, being most dense at the pharyngeal orifice At the tubal floor several folds housing groups of goblet cells (*G*). The glandular density is greatest in the rhinopharynx (*R*). PAS-stained whole moun 20

Fig. 4 Tube and rhinopharynx from 1-week foetus (CR length 163 mm) Tube cut through the roof, cartilage removed In the medial wall (*I*) there are already large branched glands which are long and have grown deep down into the submucosa (*arrows*). The glands hav only reached the middle of the tube. In the lateral (*external*) wall (*E*) the glands are smaller and flatter with fewer tubules. At the pharyngeal orifice large glands are seen also in the lateral wall. In the rhinopharynx (*R*) number of large glands, decreasing in size towards the pharynx (*P*). PAS-stained whole mount. × 10.

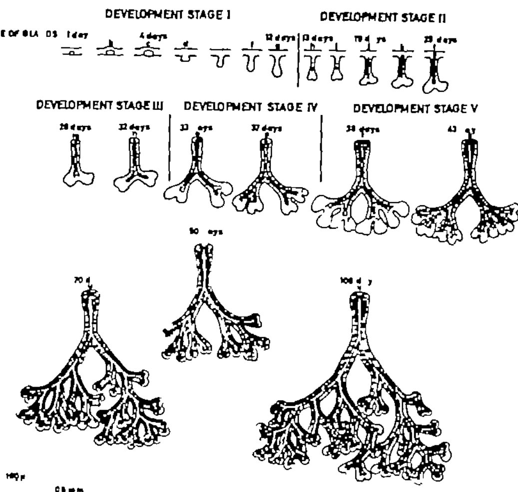


Fig 6 Development of tubal glands illustrated schematically The dark dots in the ducts and tubules indicate PAS positivity and mucin content of the cells. The age of each developmental stage has been calculated on the basis of F g. 7

cells The mucin-containing duct cells are distended and cuboid, their nuclei flat and shifted towards the base of the cell. These cells are reminiscent of the mucous cells in adult glands. None of the glands has completed its development by the 27th week they consist of tubules, but as yet there are no acini, which develop later (Fig 5)

The developmental stages described above (Fig. 6) were counted on all PAS- and PAS-Alcian blue stained tubes. By means of this differential counting of the developmental stages the growth of the glands as well as the duration and age of each developmental stage could be accurately determined (Fig. 7) The curves, which in Fig. 7 represent the number of glands belonging to developmental stages 1–5 are parallel. All curves ascend, reach a maximum of 10–15 glands, and then steadily descend towards 0 The decrease in the number of glands of the younger stage is due to the fact that the glands have grown to an older stage which therefore increase in number The distance between the curves represents the time which the glands need for developing from one stage to the next. Thus, developmental stages

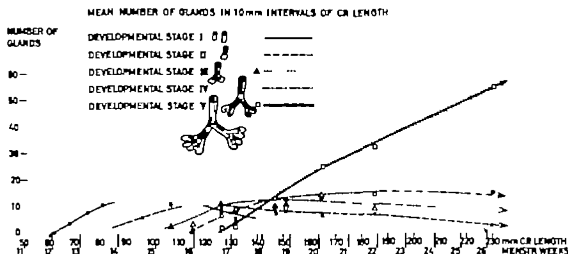


Fig 7 Number of glands in the various developmental stages. During the first weeks the number of glands of the youngest developmental stages is largest. In the 27th week most glands are of developmental stage 5 none of stage 1

1 and 2 each last for 13 days (Fig. 6) developmental stages 3 and 4 for 6 days each The first, second, third, and fourth division of the gland starts when it is 13 26 33 and 38 days old respectively The growth rate through the first 5 stages is the same for all tubal glands regardless of when and where they develop The same finding has been made in studies of the glandular development in the trachea (Tos, 1966) and bronchi (Tos, 1968 *a*)

Spread of Glands in the Tube

Development of the glands starts in the rhinopharynx and thence spreads to the tube At the beginning of the 12th menstrual week (CR length 51 mm) the first glandular primordia are found in the lateral wall of the rhinopharynx, in an area demarcated ventrally by the soft palate, cranially by the tubal orifice and caudally by the upper pole of the tonsil At this time only a few glands are present in the nasal vestibule, none in other parts of the nose (Figs. 1 2) From the 12th to the 15th menstrual week the glands spread to a larger area in the rhinopharynx—comprising also the soft palate and uvula, posterior wall of the rhinopharynx, upper half of the pharynx, Rosenmüller's fossa —and in part also towards the roof of the rhinopharynx.

At the beginning of the 13th menstrual week (CR length 62 mm) the development of glands in the Eustachian tube starts (Fig. 8) At this time the first glandular primordia are visible in the medial wall of the pharyngeal quarter (Fig. 1) By the end of the 13th menstrual week there are 6 glands in this area, none in the lateral wall (Fig. 9), where new formation of glands starts later than in the medial wall. In the 14th week the glands spread towards the second quarter of the medial wall, and gland formation starts in the lateral wall. By the end of the 14th menstrual week there is an average of 13 glands in the tube (Fig. 8) 10 of which are in the medial wall and 3 in the lateral wall —10 in the first (pharyngeal) and 3 in the second quarter In the course of the 15th and

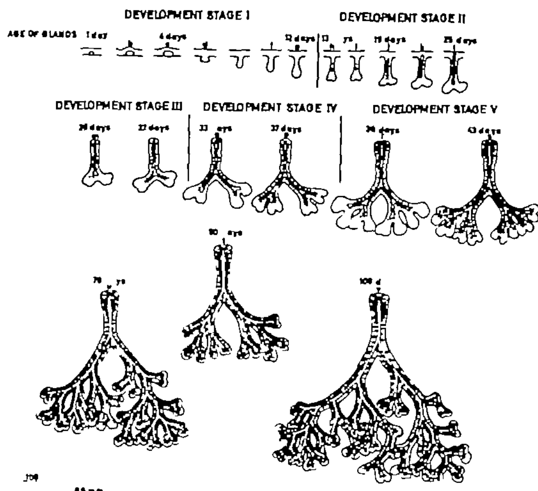


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cells The mucin-containing duct cells are distended and cuboid, their nuclei flat and shifted towards the base of the cell. These cells are reminiscent of the mucous cells in adult glands None of the glands has completed its development by the 27th week, they consist of tubules, but as yet there are no acini, which develop later (Fig. 5)

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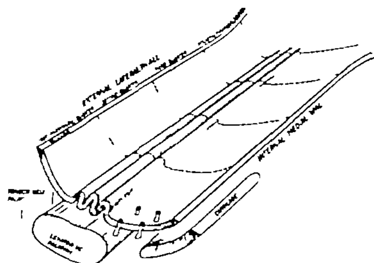


Fig 9 Situation and distribution of glands by the end of the 13th menstrual week, and shape of tube presented schematically. The tube has been cut along the roof and smoothed out. The cartilage in the lateral wall has not yet developed, and the cartilage of the medial wall has not yet reached the tympanic cartilage. The levator and tensor veli palatini muscles are well-developed. In the floor of the tube deep mucosal folds.

32, in the second quarter 22 glands. From the 19th to the 27th week the intensity of glandular new formation decreases, and in each week the increase is one less than in the preceding week, viz. 8 in the 20th week, 7 in the 21st, 5 in the 23rd, and one in the 26th week. After the 26th week there is probably no further gland formation. By the beginning of the 27th menstrual week (CR length 230 mm) the tube contains an average of 86 glands, 50 of which are in the first quarter and 36 in the second, 46 in the medial and 40 in the lateral wall.

By means of these quantitative studies of the glands throughout development (Fig. 8 and Table I) the entire developmental process may be accurately followed. Spread of glands takes place from the pharyngeal orifice to the middle of the tube, where, during the first waves in the 13th menstrual week, glands are laid down only in the first quarter (Fig. 9). By the 14th and 15th week a few glands are already visible in the second quarter (Fig. 10) but at this age the number of glands in the first quarter is 3–4 times that in the second. By the 16th and 17th week the number of glands in the first quarter is only twice that in the second. During the subsequent weeks an equal number of new glands appear in the first as in the second quarter. The same regularity was found in the trachea (Tos, 1966)—where the glands spread into the cranio-caudal direction and occur 9 days later at the carina than in the subglottis—and in the main bronchi (Tos, 1968 *a*) where a time difference of 2–3 days was also found between the onset of development in the cranial and caudal half. Although the tube is only 3 mm in length in the 13th menstrual week, the difference in time between incipient gland formation in the pharyngeal and second quarter is 10 days, and 14 days elapse from the time that the first glands are laid down in the pharyngeal orifice until they are laid down in the middle of the tube. This pharyngo-tympanic process of maturation is easily understood when considering that during its formation in the 3rd–7th week the tube is laid down as a slit-shaped pouch from the 1st visceral groove at the bottom of the foregut (Hammar 1902). Thus, the epithelium and mucosa are ripe for differentiation and newformation of glands ear

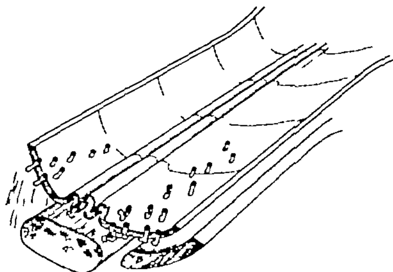


Fig 10 Situation and distribution of glands by the end of the 15th menstrual week. A few glands in the second quarter of the medial wall. Older and larger glands are seen in the first quarter where they have already reached developmental stage 2. Only a few glands as yet in the lateral wall. The cartilage of the lateral wall has not yet developed.

ller in the pharyngeal part than in the middle of the tube, and this process of maturation occurs gradually

The other difference found by means of the quantitative studies of glands is that between the onset of gland formation in the medial and lateral wall. As demonstrated, gland formation starts first in the medial wall (Figs 1 2, 9 and able I) and 10 days later the first glands may discerned in the lateral wall. By the 13th-th week the number of glands in the medial wall is three times that in the lateral wall (Fig 10 and Table I) During the subsequent weeks the number of glands in the lateral wall increases and the difference will be considerably reduced. Thus, mucosal maturation and ability for differentiation of the epithelium occur 10 days later in the lateral than in the medial wall, and the process of maturation also takes the pharyngo-tympanic direction. The question is then, whence the differentiation of the epithelium and gland formation spread to the lateral wall. Either by way of the tubal floor from the medial wall or by way of the orifice from the rhinopharynx. Several factors indicate that the lateral wall fills with glands both from the medial wall and from the rhinopharynx: As already demonstrated, glands and goblet cells form first in the lateral wall of the rhinopharynx, caudally to the tubal orifice whence they spread into the cranial direction in a way so that the tubal orifice will be encircled from behind. Thus, the wave of differentiation first reaches the lower border of the tubal orifice, Rosemüller's fossa, and posterior lip of the orifice, whence it spreads to the medial wall. Gradually the differentiation continues towards the rhino-pharyngeal roof, reaching the upper and anterior edge of the tubal orifice and thence the lateral wall. This encircling of the tubal orifice, however is a fairly slow process, and as late as the 16th week there are as yet no glands anteriorly to the tubal orifice (Fig. 2) Since at this age there are already 17 glands in the lateral wall, it is likely that these glands have spread from the medial wall. The same findings have been made in the trachea (Tos, 1966) where the spread of glands starts 9 days earlier in the membranous than in the cartilagious wall and where the gland formation to the cartilaginous wall spreads from both sides of the membraneous wall and from the cranial aspect.

DISCUSSION

The mean number of glands found in the 27th menstrual week was 86 50 of which were in the first quarter 36 in the second, 46 in the medial and 40 in the lateral wall. In the third quarter only 3–5 glands were found, none in the fourth quarter or in the middle ear The so far regular gland formation which decreases towards the 25th week and presumably ends around the 26th week (Figs. 7 8) indicates that in prematures after the 27th week and in newborns there is presumably no new formation of glands. In the trachea (Tos, 1966) and in the main bronchi (Tos, 1968 *a* Tos, 1968 *b*) there was also no further gland formation after the 25th menstrual week. The number of tracheal glands in children (Tos, 1970 *a*) and adults (Tos, 1970 *b*) is the same as in foetuses older than 25 menstrual weeks. The quantitative findings in the Eustachian tube described above will probably be found to apply also in normal children and adults

The function of the glands is to produce mucus. Since the individual glands are larger and their number greater in the pharyngeal quarter than in other parts of the tube, the main part of the secretion forms in the pharyngeal quarter The flow of mucus and the movements of cilia are in the pharyngeal direction (Zöllner 1942), and under normal conditions the mucus will be emptied into the rhinopharynx. The mucus moistens and protects the mucosa and forms, together with the ciliary function—in the same way as in the lower air ways—a barrier against infection of the middle ear

Little is known about the role of the mucous glands in the Eustachian tube under pathological conditions. In Farrior's (1943) opinion the increase in tubal mucus production was the most important factor in catarrhal infection of the tube. He found the mucous glands to be in a condition of maximum secretion. Oedema of the mucosa and mucus from these glands and goblet cells causes obstruction of the tube In chronic tubal inflammation Farrior also found increased secretory activity of the glands and goblet cells In a child with acute otitis and in 2 adults with mild otosalpingitis Aschan (1954) found inflammatory changes of the mucous tubal glands. In my opinion, an increase in the mucous secretion in the tube must be an important factor of tubal function. The 86 glands represent a considerable glandular mass which under pathological conditions yield a hyperproduction of mucus which collects in the narrow tube. Oedema of the mucous membrane and a reduced ciliary function, found in acute and chronic catarrhal conditions, prevent the normal flow of mucus towards the rhinopharynx, and this may lead to tubal obstruction.

In the present study no signs of gland formation were found in the bony part of the tube or in the middle ear and it was found probable that normally no further glands form later Studying the middle-ear mucosa by the same whole-mount methods Bak Pedersen & Tos (1970) in an autopsy material of adult persons, found mucous glands and gland-like structures in the middle-ear mucosa. The glands were found also in cases which had not clinically exhibited signs of acute or chronic diseases of the middle ear and in which the middle ear drum, and cellular system were normal. Friedmann (1963) found gland-like elements and Sadé (1966) mucous glands in the middle-ear mucosa in chronic serous otitis. In mastoiditis Friedmann and in chronic otitis media Palva et al. (1968) found glands in the mucous membrane of the mastoid process and of the middle ear Friedmann believes that under pathological conditions the middle-ear endothelium will be transformed into a columnar epithelium containing many goblet cells. In my opinion the glands arise as a link in this transformation. The question is what happens to the newly formed glands after the pathological irritant (acute purulent and serous otitis, chronic purulent and serous otitis) has ceased and the ear has again become normal. In the trachea it was demonstrated (Tos, 1970 *b*) that the number of glands remains unchanged throughout life, also under pathological conditions such as chro-

nic bronchitis. It is probable that the glands in the middle ear do not disappear when the pathological condition has ceased, but their secretory activity must become greatly reduced. In the event of a new pathological irritant the glands may again become active and produce mucus.

Thus, the entire mucus-producing system in the tube and middle ear still poses many unsolved problems. Before the pathological conditions can be studied, it is necessary to establish, on a major material—by means of quantitative studies of the glands and goblet cells—the number density and distribution of glands and goblet cells in normal children and adults and to establish the sequelae of acute, short lasting diseases of the tube and middle ear upon the mucous elements in the tubal and tympanic mucosa.

ZUSAMMENFASSUNG

Die Absicht der Arbeit ist, die Entwicklung der mukösen Drüsen in der Ohrtrompete zu klären. 47 Ohrtrompeten von 33 Foeten, die 11 bis 27 Menstruationswochen alt waren, wurden nach PAS-Alcianblau und Osmiumtetroxyd — Methoden der Ganzpräparate gefärbt und untersucht. Alle Ohrtrompeten wurden freipräpariert und gefärbt in toto: alle Drüsen wurden gezählt und untersucht. Es wurde gefunden, dass die Drüsenneubildung in der 12. Menstruationswoche in … r Seitenwand des Nasenrachens, kaudal … on Tuben- … beginnt und in die Ohrtrompete fortsetzt. Im ersten Teil der 13. Menstruationswoche beginnt die Drüsenneubildung in der Ohrtrompete, zuerst in der Lamina interna, 10 Tage später in der Lamina externa. Die Drüsen werden zuerst in dem pharyngeal Ostium angelegt und werden gradweis in die tympanale Richtung ausgebreitet, so dass sie nach 14 Tagen die Mitte der Ohrtrompete erreichen. Bis zur 17 Menstruationswoche entstehen wöchentlich ungefähr sieben, on der 17 bis 19 Woche ungefähr neun Drüsen. In den nachfolgenden Wochen nimmt die Intensität der Drüsenneubildung gradweise ab und nach der 26 Woche werden wahrscheinlich keine neuen Drüsen mehr gebildet. Im 27 Woche gibt es in der Ohrtrompete durchschnittlich 86 Drüsen, wobei 50 in dem ersten (pharyngealen) Viertel, 36 in dem zweiten Viertel der Ohrtrompete liegen. In der tympanalen Hälfte der Ohrtrompete gibt es keine Drüsen. In der Lamina interna gibt es durchschnittlich 46 Drüsen, in der Lamina externa 40 Drüsen. Mit Hilfe der kvantitativen Untersuchungen wurde auch die Entwicklung und das Wachsen der einzelnen Drüsen illustriert.

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SECRETORY OTITIS MEDIA AND THE NATURE OF THE MUCOCILIARY SYSTEM

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Abstract. Secretory otitis media is essentially a post inflammatory reaction of the middle ear and involves excess mucus production by the middle ear lining. This lining consists of mucociliary system, and in analogy to situations in other respiratory epithelia, its clearance action is deficient in S.O.M. Thus, the nature and function of the mucociliary system and its relation to factors such as ventilation of the middle ear are key questions in this disease. Observations on the clearance action of the mucociliary system in animals indicate the role of mucus as a coupling agent translating ciliary beat into effective clearance. Further observations on the nature of mucus suggest the specific properties, making it perform this function, as being mainly due to the crosslinked or entangled hydrophilic glycoproteins. The existence of such network may impart to the material both its unique rheological characteristics and the solubility behaviour necessary for its specified role. It is suggested that possible reason for clearance failure in pathological situations is an alteration in the macromolecular structures and thus in the ability of the mucus to perform as in normal systems.

The treatment of secretory otitis media (S O.M.) has been of great disappointment to us in view of the number of recurrences (Proud, 1968 Stevens, 1958) It seems to us that S.O M. is essentially a post-inflammatory middle ear reaction and one of the main problems confronting us today is the clearance of the effusion which has accumulated in the middle ear—which in turn depends to a great extent on the middle ear mucociliary system.

We would like to offer our observations pertinent to this point of view and our experiments on the mucociliary system which might lead to a better understanding of factors governing clearance from the middle ear

The clinical picture

The pathogenesis of S O.M. was thought at one time to be due to adenoids obstructing the Eustachian tube and thereby causing air absorption from the middle ear and accumulation of a non-inflammatory transudate secondary to the negative pressure thus formed (Lawson, 1927) Verification of this postulated mechanism was seen in the retracted tympanic membrane, in the presence of adenoids, and in the alleviation of the syndrome after their removal. Variations of this basic concept are based on the belief that the Eustachian tube itself is obstructed secondary to some inflammatory or allergic reaction (Jordan, 1949). According to all these theories the effusion in the middle ear which is often of a viscous character is a fluid of the transudate type (Wright & Kapadia, 1969 Carlson & Lökk, 1955) (Absorption of water and concentration of the effusion is looked upon as the cause of turning a thin, low viscosity transudate into a viscous material.) This effusion was not regarded as being actively secreted from the middle ear lining which *a priori* was not regarded as a true secreting mucosa (Sachs, 1952)

As interest in this condition arose, data became available which threw some doubt on



Fig 1 Ciliated columnar (pseudostratified) epithelium from the lining of a normal middle ear ×200.

the validity of the classical "ex vacuo" theories. Thus a significant number of patients show middle ear effusion with no adenoids in their nasopharynx or show the condition recurring after adenoidectomy (Davison, 1958) we have found such recurrences in 35% of our cases Furthermore, chemical analysis of the middle ear effusions showed a large quantity of proteins, glycoproteins and nucleoproteins, indicat an inflammatory mucus-containing exudate ot compatible with a transudate (Senturia, 1960)

Support that appearance of middle ear effu sions is secondary to a regional inflammation is found in experiments in dogs in which the Eustachian tube was cauterized, resulting in a middle ear inflammation which subsequently also showed serous or mucoid effusion (Sadé et al., 1959), no obstruction of the Eustachian tube being observed histologically Previous studies by Flory (1954) have shown that inflammatory reactions of mucus membranes are characteristically followed by excess produc tion of mucus and subsequent hyperplasia of *mucus-forming elements. Obvious upper respi*ratory infection, colds" or otitis media of one sort or another preceded the syndrome (at some time) in 87% of our young patients, and in 38% of the adults confirming previous clinical impressions by Stevens (1958)

Open questions

The question therefore arose whether the middle ear is really devoid of mucus-forming elements. In studies motivated by this question, it has been shown by Sadé (1966 *a*) and Lim et al. (1967) that the middle ear is in fact partially lined with a fine mucosa which bears cilia (Fig. 1) and secretes mucus.

Biopsies of middle ear mucosa from S.O.M. patients as well as histological examination of autopsies have demonstrated the presence of impressive hyperplasia of mucus-producing elements (Fig. 2) and hypersecretion of mucus (Sadé, 1966 *b* Friedman, 1963 Bendek, 1963) The same findings have been reported in chron ic otitis media by Sadé & Weinberg (1969)

The clinical data is thus supported by biochemical and histological evidence which indicates that the middle ear effusion originates in situ, secondary most probably to an inflammatory process and is in essence a mucoexu date of one form or another

Further studies (Sadé, 1967) of the cilia in the middle ear have provided direct evidence for ciliary clearance action, explaining previous

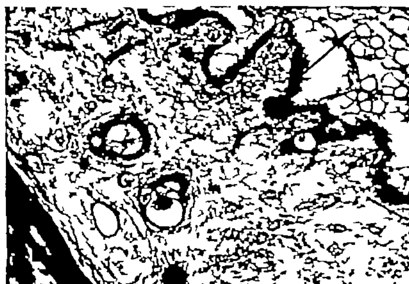


Fig 2. Microscopic section from an autopsy of a patient with S.O.M. *G* enlarged mucus glands staining PAS positive in thickened mucosa, *B* bone. Arrow denotes PAS-positive mucus covering epithelium and exuding into middle ear cavity ×80.

studies by Compere (1958) with radio opaque material indicating the natural mode of exudate clearance from this cavity

The favourable and prompt therapeutic response to various grommets (e.g. Feuerstein, 1966) inserted into the middle ear in S.O.M. indicates that the ciliary system in this condition still functions, and provides further evidence for the patency of the Eustachian tube. Thus retention of the effusion or lack of clearance is reversed upon aeration of the system. Whether aeration of the middle ear with the help of grommets does essentially provide for better clearance or suppresses the stimulus needed for continuous mucus secretion is an open question.

It seems that although actual hermetic obstruction of the Eustachian tube is not found in S.O.M., aeration of the middle ear may be defective. Quantitative measurements of aeration (oxygen to CO_2 relation) and normal pressure gradients in the middle ear are so far not available, although several attempts have been made to obtain this information (Koch et al. 1963 Flisberg, 1966) Existence of an actual negative pressure in the middle ear in cases of S.O.M. does not however seem likely as the effusion can be seen to spurt out in many cases when the drum is incised under water while a suctioning effect into the middle ear when paracentesis is done with water covering the drum does not occur (Sadé, 1966 *b*)

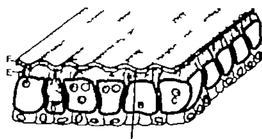


Fig 3 Schematic representation of the mucociliary system. *B* ciliated cell, *C* mucus granule *D* goblet cell ejecting mucus towards mucus blanket *E* cilia *F* mucous blanket.

The mucociliary system

The fundamental open questions with regard to S.O.M. concern the mechanism governing the clearance of the effusion from the middle ear by cilia and possibly the specific stimulating factors governing mucus secretion, as well as their relationship to aeration of the middle ear. Ciliary action in the middle ear is similar to that found in the lungs and sinuses or in the digestive tract of lower animals such as frogs,

and in marine animals, all of which are covered by a mucociliary system (Fig. 3) Indeed, several conditions such as asthma, chronic bronchitis and mucoviscidosis cause the bronchi in the lungs to be clogged with mucus—and the sinuses show similar problems in sinusitis, thus these situations are analogous to that of the ear in S O.M. and the primary questions of mucus clearance by cilia apply to them as well. Since in all these conditions mucus is secreted in excessive amounts and is not cleared, the question arises whether changes in the quality of the mucus could account for these phenomena.

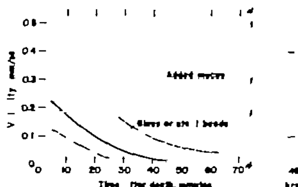


Fig. 4 Velocity of transport of foreign particles and of added mucus on frog and toad palates, as function of time after death. —— average of 30 experiments; – – –, range of velocities observed.

The role of mucus

Cilia have been known to beat metachronally (Satir 1963) and propel a mucus blanket with the particles adhering to it, thus bringing about clearance Cilia have also been known to continue beating in vitro for days after the death of the animal (Dalhamn, 1956 Hilding, 1957) However in this case their clearance action terminates before they stop beating. In our laboratory (Sadé et al., 1970) we have found that in such preparations, when deposited foreign bodies are no longer cleared but the cilia still beat, active clearance can be resumed if mucus is placed on the surface of the preparation (Fig. 4) The stage when foreign materials will no longer be cleared by cilia, but mucus will, is termed a state of mucus depletion. It is the cessation of mucus secretion to above the tips *of cilia which prevents clearance* Once the "coupling" mucus is made available, clearance will continue for a long time. The weights of foreign bodies which can be cleared by such a mucosa, by its own mucus or by added mucus, vary within a wide range and clearance proceeds independently at a constant speed (for a frog palate, at a rate of 1 mm/3–5 sec)

Thus it is seen that mucus functions not merely as a protecting and lubricating sheath (Gottschalk & Graham, 1966) but also as a coupler necessary for the translation of the ciliary beat into mechanical, directed clearance. It therefore has to possess specific properties to enable it to perform this task. The specific properties of mucus are to be sought in its molecular structure and the mechanical properties imparted to it by this structure. It is important to understand the nature of these specific properties and their origin, and to find out to what extent their alteration in pathological situations is responsible for failure of mucociliary interaction and clearance.

Mucus and its properties

Mucus consists of about 98% water plus salts and proteins. The proteins are mainly albumin γ-A globulin and epithelial-type glycoproteins with small amounts of various other blood proteins and enzymes (Schultze & Hermans, 1966) The mechanical properties of mucus are imparted to it by its glycoproteins (Gibbons, 1959) which are very high molecular weight polyelectrolytes consisting of long protein chains with oligosaccharide side chains terminating with sialic acid (Gottschalk & Graham, 1959) In a study of the specificity of mucus action (Sadé et al., 1970) we tried to replace mucus by water soluble long chain polymers having similar properties, with regard to viscosity elasticity and spinnability We did not find any system which acted like mucus when added to depleted tissue. Rather than enhance clearance, such substitutes converted the metachronal ciliary rhythm into a synchronous or arhythmical beat, which was ineffective in causing clearance.



Fig. 5. Microscopic section from a frog palate showing typical mucociliary system with goblet cells (*G*) emitting mucus which forms a PAS-positive mucous blanket (*arrow*) on top of the cilia (*C*). × 400. These structures are alike in the mucociliary lining of mammalian respiratory organs and middle ear. *Note:* no mucus between the cilia.

A possible explanation for this phenomenon may be found in the dimensional and structural relationships of the system. Cilia are about 7 μm long and 0.3 μm wide with distances of 500–1 000 Å between them (Satir 1965). Should a high molecular substance as used by us, penetrate the spaces, ciliary rhythm and motion might be impeded by adhesive or viscous forces. Indeed, when the mucociliary system is investigated histologically no mucus is seen in the interciliary spaces (Fig. 5). How then does mucus pass from the secreting cells to above the ciliary tips? Microscopic examination of the mucociliary system shows that mucus passes directly from the goblet cells (which are interspaced between relatively large areas of cilia) in narrow streams to the mucus blanket (Fig. 5) and is not present homogeneously throughout the ciliary spaces.

We know therefore, that mucus does not normally mix with the interciliary fluid and that polymers which do so disturb the ciliary rhythm, and prevent the translation of their motion into clearing action. Are the mucus molecules too large to penetrate the ciliary interspaces or is there some other mechanism involved in keeping mucus from penetrating the interciliary spaces?

The glycoproteins of mucus contain a large number of saccharide units which are very hydrophilic due to their hydroxyl groups. Indeed mucus when dried will reabsorb water easily and swell. Still, mucus does seem to be a true solution, or a simple fluid, as it has been often referred to (Wright & Kapalis, 1969). Numerous attempts to dissolve mucus in water or saline solution show the presence of a network in mucus which withstands dissolution for many days. Part may dissolve but the main network remains intact. Bond breaking agents such as urea and dithiotreitol will hasten the breaking up process. Ultrasonication, drastic proteolytic treatment or hydrolysis are found to act more efficiently in liquefying or dispersing mucus.

The failure of mucus to form molecularly disperse solutions excludes it from the class of simple fluids, while at the same time it cannot be included in the group of solid materials since it changes its shape easily under stress. Mucus therefore may be regarded as a semi solid or gel the constituents of which, the glyco-

proteins, while hydrophylic, are at the same time crosslinked or entangled (Davis & Dippy 1969) to form supermolecular structures. This classifies mucus as similar to structural proteins and provides an explanation why it does not enter the ciliary interspaces as well as why it is not dissolved from eel skin (Negus, 1963) or clam gills (Gray 1928) Indeed, if mucus were soluble in water all these as well as many other biological functions of mucus would not be possible.

The crosslinked structure of mucus explains also some of the difficulties encountered when studying it, such as its immobility in electrophoresis (Neuhaus & Moghissi, 1962) and in the ultracentrifuge (White & Elmes, 1960) or the difficulty of measuring its rheological properties (*Wells et al., 1961*).

The specificity of mucus is therefore probably a reflection of several parameters of its macromolecular arrangement which govern its rheological and physiological properties (Denton et al., 1968) Familiarization with the biochemical composition of the various constituents of mucus and their interaction to form the specific spacial arrangement (of which crosslinkage is only one facet) might lead us to an understanding of the nature of mucus in its relation to cilia.

ZUSAMMENFASSUNG

Die sekretorische Otitis (S.O.M.) ist im wesentlichen eine Reaktion des Mittelohres auf eine (vorangegangene) Entzündung und äussert sich in einer vermehrten Schleimabsonderung im Mittelohrbereich. Dieser Trakt besteht aus einem Schleimhautsystem und analog zu ähnlichen Erscheinungen in anderen respiratorischen Epithel-Zellen ist bei S.O.M. der Prozess der Schleimabführung gestört. So sind Natur und Funktion dieses Schleimhautsystems und seine Beziehung zu Faktoren wie die Ventilation des Mittelohres Schlüsselfragen bei dieser Krankheit. Die im Mucus-System von Tieren beobachteten Vorgänge bei der Schleimabsonderung deuten darauf hin, dass der Schleim die Rolle einer Kopplungssubstanz spielt. Dabei ist das dynamische V rhalten der Flimmerhärchen (Cilia) Basis für einen wirksamen Mechanismus der Schleimabführung *Weitere Beobachtungen an* den Schleimstoffen führen zu der Schlussfolgerung, dass deren spezifische funktionelle Eigenschaften hauptsächlich auf der kreuzweise verbundenen, mehrfach verstrickten Struktur basieren. Ein derartiges Netzwerk kann der Substanz sowohl *einzigartige rheologische* Eigenschaften verleihen als auch das für seine spezielle Rolle notwendige Löslichkeitsverhalten bedingen. Es wird vermutet, dass ein Versagen des Schleimabführungsmechanismus in pathologischen Fällen möglicherweise auf Veränderungen der makromolekularen Struktur des Schleims zurückzuführen ist, wobei seine normale Funktionsweise beeinträchtigt wird.

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PENICILLIN CONCENTRATION IN MIDDLE EAR SECRETION IN OTITIS

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Abstract. The author has examined the penicillin concentration in otitis and the relationship between penicillin concentration in serum and ear secretion. The series consists of 273 middle ear samples; 206 of these were acute cases, 22 catarrhal, and 45 chronic. Serum samples totalled 142. The results are presented by tables which show the following: In *acute otitis* penicillin can be noted in the middle ear secretion as early as half an hour after parenteral administration of the drug. The penicillin concentration is highest 1 hour after administration. Penicillin disappears more slowly from the ear than from serum. In *catarrhal otitis*, penicillin appears very little in the ear secretion despite high serum concentrations. In *chronic otitis*, somewhat higher penicillin concentrations were oted than in the catarrhal inflammation. Compared th acute otitis, the concentrations were, however w,

Compared with the wide research work carried out in order to determine the serum concentration of different penicillin preparations, investigations of tissue concentrations are remarkably sparse and have been made with smaller materials. One reason may be the relative ease of blood sampling compared with the greater difficulty sometimes even impossibility of sampling live human tissue. The present investigation examines penicillin concentrations in the infected middle ear and attempts to throw light upon the following aspects. (1) relationships between the penicillin concentrations in serum and ear secretion, (2) penicillin concentrations in ear secretion in (*a*) acute otitis, (*b*) catarrhal otitis, (c) chronic otitis.

MATERIAL AND METHODS

The material has been collected from service men with otitis Military Hospital 2, Turku Finland. Thus, it is homogeneous as to age and physical fitness. The number of ear samples was 273 206 of these were acute, 22 catarrhal, and 45 chronic otitis cases. 142 blood samples were taken, 89 in connection with acute, 22 with catarrhal, and 31 with chronic otitis

Before taking the samples, all patients received an intramuscular injection of penicillin. The amount of penicillin, 400 000 units of Di-penita preparation (Lääke Oy Turku Finland) was the same for all patients. (Procain G-penicillin 300 000 units + Sodium G-penicillin 100 000 units.)

Every patient was examined and treated by the author himself. Sampling technique and distribution into different groups of otitis were thus the same for all patients Ear samples were taken by aspiration of punctured middle ear cavity This method has been reported earlier in this periodical (Lahikainen, 1953) The samples of chronic otitis were taken by sterile aspiration from middle ear 30 min, 1 hour 2–4 hours, 6 hours, and ≥ 12 hours after the penicillin injection. They were immediately transferred to the Department of Medical Microbiology at Turku University where penicillin determinations and bacterial examinations were

Table I. *Average penicillin concentration in units/ml range relationship between positive samples and the total of samples after a penicillin injection of 400 000 units i.m. acute otitis*

	Hours after administration of penicillin									
	1/2		1		2–4		6		> 12	
	Ear	Serum	Ear	Serum	Ear	Serum	Ear	Serum	Ear	Serum
Average, units/ml	0.38	2.37	1.27	3.58	0.98	1 60	0.33	0.30	0.17	0.03
Range, units/ml	2.4–0	5.0–0.5	8.2–0	11.2 1.2	8.8–0	8.1–0.3	0.8–0	1.5–0	0.3–0	0 4–0
Positive samples of the total of samples	9/18	7/7	65/83	50/50	51/65	29/29	9/15	9/12	7/18	1 12

carried out. The penicillin determinations were performed according to the agar-cup method, as described in Groves & Randall, *Assay Methods of Antibiotics*. The test strain was the international strain *Staphylococcus aureus* 209 P

RESULTS

Acute otitis

The number of the examined ear samples was 206 and that of the blood samples, 89 The results are presented in Table I and show that as early as 30 min after the administration of penicillin, it is evident in the middle ear secretion. The penicillin concentration is highest after 1 hour in both ear secretion and serum. After this, the penicillin concentration decreases—more slowly in the ear secretion than in serum. The number of positive samples underlines this same fact. First, only a half of the ear samples are positive. After 1–4 hours, the majority of the ear samples are positive. After 6 hours, the number of positive samples and the degree of penicillin concentration are about equal in both groups. After > 12 hours, the penicillin concentration is higher in the ear secretion than in serum. The number of positive samples shows the same 7 out of 18 ear samples were positive whereas only 1 of 12 serum samples was positive

The majority of the samples were taken 1–4 hours after the administration of penicillin. On this basis, it can be concluded the higher the average penicillin concentration in serum, the higher it is in th ear secretion as well. The highest penicillin concentrations, 8.2–8.8 units/ml, were present in the ear secretion after 1–4 hours. The highest concentrations in serum during the corresponding period were 11.2–8 1 units/ml.

Catarrhal otitis

In this investigation, catarrhal otitis means otitis whose main symptoms are blocking of the ear and impaired hearing. Many patients also report that they have "water in the ear" whereas pain is not one of the symptoms of this kind of otitis. The tympanic membrane is opaque, not reddened or bulging. The ear secretion is not purulent or haemorrhagic; it is either thin and serous or viscid and mucous.

Table II shows that the penicillin concentration of the ear secretion in this kind of otitis is almost nil. In as few as 6 cases out of 22, there was penicillin, and in all cases, less than 0 1 units/ml In all 22 cases, penicillin could be found in serum. The range of the penicillin concentration in serum was 7 1–0.3 units/ml. Bacteriologically all ear samples were sterile.

Chronic otitis

Compared with acute otitis, the penicillin concentration of the ear secretion in chronic otitis is very low

Table III shows that 38 samples out of 45 contained no penicillin at all. The number of so-called trace samples (< 0 1/ml) was 6. The highest penicillin concentration in the ear secretion was 0 5 units/ml. In acute otitis, the corresponding figure was 8.8 units/ml

Table II *Penicillin concentration in units /ml in catarrhal otitis*

1 hour post admin.			2-4 hours post-admin.		
Patient	Serum	Ear	Patient	Serum	Ear
A. P	[illegible]	0	S. I.	[illegible]	0
A. K.	7 1	Trace*	A. R.	[illegible]	Trace
M. S	3.6	0	U A.	1.3	0
A. L.	5 6	0	R. P	1.3	Trace
O K.	2.0	0	N. Y	1.5	Trace
V S.	2.8	Trace	P A.	0.3	Trace
A. V	[illegible]	0	O K.	2.8	0
L K.	[illegible]	0	J V	1.5	Trace
P A.	2.6	0	L. K.	0.9	Trace
A. K.	4 7	0			
A. S.	4.2	0			
S. V	1.6	Trace			
A. V	2.3	0			

Trace: penicillin concentration <0 1 units/ml.

Bacteriological examination showed *10* sterile samples of which 5 cases had a penicillin concentration *in the ear secretion* of 0.2–0 4 units/ml. In the majority of the samples, a bacterium species could be isolated, the most common being *Staphylococcus aureus* and *Pseudomonas aeruginosa*. These types most often cause chronic otitis.

DISCUSSION

In the results examined, attention is paid to [illegible]e fact that the penicillin concentration of [illegible]e ear secretion is many times higher in acute than in chronic otitis. The difference is accentuated if acute and catarrhal otitis cases are compared.

In the pertinent literature, the author has not found publications dealing with penicillin concentrations in chronic otitis As to catarrhal otitis, some investigators (Motokawa, 1957 Silverstein et al., 1966) state that penicillin does not enter the ear secretion at all, or it appears in too small quantities to be measured.

What are the causes of the low or non-existent penicillin concentration in the ear secretion in catarrhal and chronic otitis? The type of the infection can be considered one factor In catarrhal otitis, the inflammation is mild, caused by viruses and not by bacteria (Berglund et al., 1966). Catarrhal otitis may also derive from an allergy Thus, capillary permeability increases less in catarrhal otitis than in severe bacterial infections. This results in the considerable or even complete inhibition of penicillin transport from the capillaries to the middle ear

Even in chronic otitis, the infection is milder and more long-term, and does not increase the permeability of capillaries as much as does acute otitis.

Further penicillin may possibly bind the proteins in the ear secretion. According to some investigators (Hoople, 1950 Tremble, 1951 Senturia et al., 1958 Voori, 1959), the amount of protein in the ear secretion in catarrhal otitis is greater than in acute infections. The longer the duration of symptoms of catarrhal otitis, the greater is the total of protein content in the ear secretion.

In chronic otitis, however the low penicillin concentration is mainly caused by the bacteria that appear in chronic otitis. These are penicillinase-producing, and thus destroy penicillin.

The practical conclusion of this investigation is that penicillin is of no use in the treatment of chronic or catarrhal otitis.

ZUSAMMENFASSUNG

Der Verfasser hat die Penizillinkonzentration im Ohrsekret bei Otitis untersucht. Das Material besteht aus

Table III *Penicillin concentration in units/ml and bacteriological finding in the middle ear in chronic otitis*

1 2 hours post-admin.				4-6 hours post-admin.				>12 hours post-admin.			
Patient	Serum (units/ml)	Ear (units/ml)	Bacterio-logical finding	Patient	Serum (units/ml)	Ear (units/ml)	Bacterio-logical finding	Patient	Serum (units/ml)	Ear (units/ml)	Bacterio-logical finding
E. R.	1.6	0.3	Sterile	A. M.		0	*S. aureus*	H. A.	0	0	*Pseudomonas*
K. H.	4.2	0.2	Sterile	A. L.		0	*Pseudomonas*	L. M.	2.2	0	*S. aureus*
E. T	2.8	0	Sterile	A. L.		Trace	*Pseudomonas*	E. S.		0	*Pseudomonas*
H. M.	6.8	0.3	Sterile	K. M		0	*S. aureus*	E. S.		0	*Pseudomonas*
A. L.	1.7	0.4	Sterile	O. T	0.8	0	Sterile	M. V	0.3	0	*S. aureus*
V. L.	10.2	0	Sterile	H. H.	0.8	Trace	*Pseudomonas*	A. J	0.2	0	*Pseudomonas*
A. A.	0.6	0.4	Sterile	E. L.	0.4	0	*S. aureus*	L. K.		0	*S. aureus*
I. L.	0.8	0	Sterile	H. K.	0.6	0	*S. aureus*	B. S.		0	*S. aureus*
P. V	3.2	0.5	*Proteus*	K. K	0.8	Trace	*S. aureus*	M. S.		0	*S. aureus*
P. V	3.2	0.3	*Proteus*	V. J.		0	*Pseudomonas*				
A. S.	0.6	0	*Pseudomonas*	P. K.		0	*Pseudomonas*				
R. S.	1.1	Trace*	*Pseudomonas*	M. V	0.5	0	*S. aureus*				
O. O.	0.7	0	*Pseudomonas*	A. P	0.3	0	*Proteus*				
H. K	1.7	0	*Pseudomonas*	S. L.	0.8	Trace	Sterile				
L. L	1.1	0	*Pseudomonas*	M. K.	0.5	Trace	*S. aureus*				
A. J		0	*Pseudomonas*								
L. N	0.9	0	*Pseudomonas*								
M. T	1.7	0	*Pseudomonas*								
V. M.		0	*S. aureus*								
T. V		0	*S. aureus*								
E. T	3.5	0	*Klebsiella*								

Trace: penicillin concentration 0.1 units/ml.

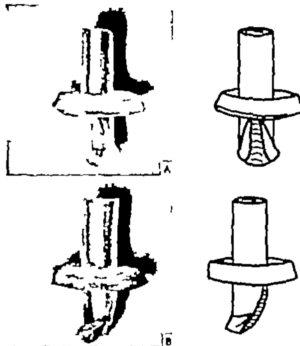


Fig 1 Photograph and schematic drawing of the modified polyethylene tube (approx. × 10). (*A*) anterior view (*B*) lateral view

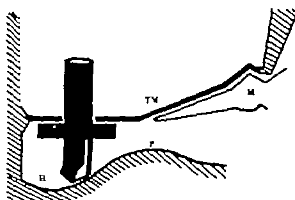


Fig 2 Schematic drawing of the modified polyethylene tube in place in the tympanic cavity *TM* tympanic membrane; *M* malleus; *P* promontory *H* hypotympanum.

ectomy and bilateral myringotomy with introduction of standard plastic tubes. There was improvement in hearing with normal audiometric curve but, 6 weeks following interven tion, the hearing deteriorated and the tubes found to have been spontaneously ex ed

For the subsequent 2 $^1/_2$ years the patient required five additional interventions with introduction of various available tubes. Conventional supportive conservative therapy was administered. There was always immediate re sponse to the treatment, but only for very short periods of time. Eventually further introduc tion of plastic tubes became impossible be cause of formation of adhesions between the drum and the promontory At this stage the bilateral loss of hearing was at the 40–50 dB level.

Finally at the age of 9 $^1/$ (1 / years ago) the author s modified polyethylene tubes were introduced in the manner described. The hear ing improved immediately and after it had remained normal for 1 year without any evidence of formation of new adhesions, the tubes were removed. The eardrums healed easily At present, 6 months after removal of the tubes and with eardrums closed, hearing remains normal.

COMMENT

A few stubborn and unresponsive cases of serous otitis media—among many hundreds easy to manage—present a serious problem, which several authors have tried to solve with different materials, surgical interventions and mechanical devices (Siirala, 1964 Silverstein, 1966, 1970 Turner 1967 Wilson, 1969) The fact that so many methods have been tried proves that none of them adequately fulfills its purpose

The result in the case reported, obtained with a modified plastic tube is encouraging. The device described provides an additional and, as we believe, valuable aid in the treatment of these, fortunately rare, but problematic cases.

ZUSAMMENFASSUNG

Ein modifiziertes Polyethylenröhrchen, welches für dauerhafte Lüftung der Paukenhöhle sorgt und gleichzeitig auch die Bildung neuer Adhäsionen zwischen dem *Trommelfell und dem Promontorium* vorbeugt, wurde beschrieben. Über den typischen Fall einer rückfälligen Otitis media serosa, durch Adhäsivprozess

kompliziert, wird berichtet. Dieser Fall wurde zuletzt mit Hilf des modifizierten Polyethylenröhrchens erfolgreich behandelt.

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STAPEDIUS MUSCLE REFLEXES AND OTO-NEUROLOGICAL EXAMINATIONS IN BRAIN STEM TUMORS

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Abstract. Stapedius-muscle-reflex examinations were performed on two patients shown to have brain stem tumours. The reflexes were tested with an impedance-measuring device capable of measuring the stapedius reflexes not only by contralateral stimulation but with homolateral stimulations as well. In these patients, the contralateral stimulation produced no response, while homolateral stimulation resulted in normal reactions. *These indications can only be explained by* an interruption of the reflex arch of the stapedius muscle at a point within the brain stem, between the cochlear nuclei on the one side and the facial nucleus on the other side. An apparatus of the type mentioned above is valuable in the diagnosis and localization of brain stem affections and in the further investigation of the stapedius muscle reflex arch.

The stapedius muscle reflex may be elicited in normally-hearing persons by acoustic stimula ıı at intensities of more than 60–70 dB The response is bilateral, i.e the stimulation one ear causes a contraction of the stapedius *on both sides. The probable reflex arch* is sketched in Fig. 1

The afferent part of the reflex arch involves the cochlea and the acoustic nerve. The reflex centre is presumably located in the distal part of the pons (superior olivary nucleus) while the efferent part of the reflex arch consists of the facial nerve to the stapedius muscle (Jepsen, 1963)

Lack of a reflex response may be due to pathological changes in the afferent part of the reflex arch (moderate or severe hearing loss) or in the efferent part (facial palsy or middle ear disturbances) Finally a lesion in the brain stem may cut the reflex arch.

In itself, the absence of the stapedius muscle reflex upon the usual contralateral stimulation will not provide much information about the site of the interruption.

Neergaard et al. (1965) evaluated an impedance-measuring device which was able to detect reflex responses not only in the opposite *(contralateral)* ear *but also in the stimulated,* homolateral ear

Comparison of the stapedius muscle reflexes elicited by homolateral and contralateral stimulation increases significantly the possibility of making a topical diagnosis of a disruption of the reflex arch. The following examples will *illustrate this point.*

Case Report

Case I

A 49-year-old male patient was referred to the *neurosurgical department with a complaint of* numbness in the left hand and foot which had *persisted for about 9 months During the last* 3 months, he had noticed anaesthesia of the left side of the face and tongue as well. Further he suffered from double vision when looking to the left, dizziness, headaches, and reduced strength in the extremities on the right side. During the most recent month, hearing on the *left side* had been impaired, and there were periods of tinnitus.

Results of the neurological examination

There were reduced abdominal reflexes on the right side, spastic hemiparesis, ataxia, dysdiadochokinesis and impaired sensibility in the

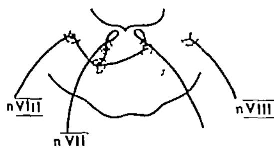


Fig. 1. Cross section of the distal part of the pons. The probable course of the stapedius-muscle-reflex arch is indicated.

right side extremities. Romberg's test disclosed deviation and a tendency to fall to the right. An eye examination was normal, with the exception of some uncoordinated movements of the eyes. There were no indications of increased intracranial pressure. X ray examination of the skull, the temporal bones—and arteriography of the internal carotic and vertebral arteries on the left side disclosed no abnormalities.

Results of the oto-neurological examination

The sensibility of the left side of the face was almost absent, facial palsy and a paralysis of the chewing muscles on the same side were also nearly total. The taste sense was residual on the anterior two-thirds of the tongue, and the naso-lacrymal reflex on the left side was severely impaired, indicating a lesion of the left facial nerve central to the geniculate ganglion.

The ear drums were normal. A slight hearing impairment of perceptive type was found in the left ear. Fowler's recruitment test was negative. Speech audiometry revealed a very pronounced discrimination loss (64 %) on the left side (Fig. 2). Békésy audiometry did not show any tone decay using continuous and intermittent tone. When the examination was repeated with simultaneous masking of the right ear, a very pronounced tone decay occurred with a continuous tone, especially at 4 000 Hz, but also at 2 000 Hz and 1 000 Hz (Fig. 3).

Caloric test showed normal reactions on the right side, and a total lack of reaction of the left side. In all head positions, a spontaneous nystagmus to the right ("destruction nystagmus") was observed.

Stapedius muscle reflexes could not be demonstrated on either the right or the left ear when stimulating the opposite ear. However, a reflex response was evident on the right side but not on the left during stimulation of the homolateral ear (Fig. 4).

Surgery disclosed a tumour in the posterior cranial fossa, lying in front of the pons, medial to the facial and acoustic nerves. The tumour seemed to invade the brain stem, and could not be removed radically. Histological examina

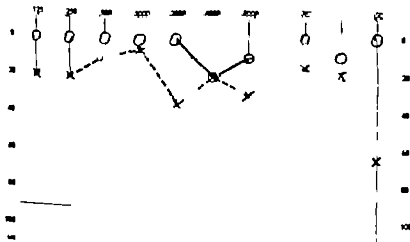


Fig. 2. Pure-tone and speech audiometry results.

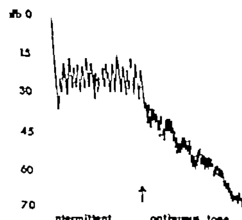


Fig 3 Békésy audiometry at 4 000 Hz, with continuous and intermittent tone and contralateral masking.

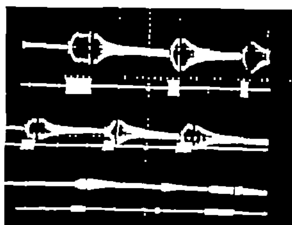


Fig 4 Stapedius-muscle-reflex on the right side, using homolateral stimulation at 95 dB, 90 dB and 85 dB. (Case 1.)

tion indicated that it was a malignant endothelio-sarcoma, probably *originating from the* meninges. The patient died subsequently An topsy was not permitted.

Case 2

A 48-year-old male patient complained of lack of coordination in the right arm, and unsteadiness when walking. Seven years previously his left kidney had been removed because of hyper-nephroma. Later metastases to the right lung were found, necessitating partial resection the lung.

…lts of the neurological examination

Pronounced ataxia of the lower extremities and a slight ataxia of the right arm was found. The deep reflexes were all hyper-active, especially on the right side On the left side, the plantar reflex was atypical, and the Chaddock reflex positive Dysaethesia was found on th. radial part of the right forearm and hand

Paresis of the conjugate movements of the eyes to the right was found. Echo-encephalography and X-ray examinations of the skull and the temporal bones indicated no abnormalities.

Results of the oto-neurological examination

No cranial nerve lesions were present. Hearing appeared to be normal. pure-tone and speech audiometry produced normal indications. While the tone-decay test (Sφrensen, 1962) showed little decay in tests of the right ear (Type 2), a pronounced tone decay of Type 3 was found in the left ear Masking of the opposite ear did not make any difference in these results

The caloric test, according to Hallpike's method revealed no time difference, but while the provoked nystagmus to the right was completely normal, the nystagmus to the left did not appear Instead, a continuous maximal deviation of the eyes to the right was seen to persist for 3 minutes.

The acoustical reflexes of the stapedius mus-

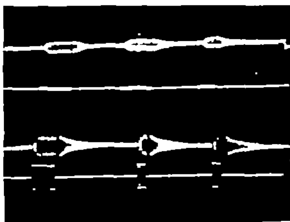


Fig 5 Stapedius-muscle-reflex on the right side, using homolateral stimulation at 80 dB and 100 dB levels. (Case 2.)

cles were normal with homolateral stimulation, but the reflexes were totally absent when the stimulus was applied to the opposite ear (Fig. 5)

The patient died one-and-a-half months later at another hospital. Autopsy revealed metastases in the cerebral hemispheres, and one metastasis on the pons (pars tegmentalis pontis) prominating slightly into the fourth ventricle. Histological examination was not carried out.

DISCUSSION

Brain-stem tumours often cause many varied symptoms, due to involvement of cranial nerve nuclei, and the ascending and descending pathways. The symptomology may be very complex, and in all cases, every symptom may be of value in diagnosing the exact site of a pathological process. In this paper we have described two cases where the stapedius-muscle reflex studies did reveal an interruption of the stapedius-muscle-reflex arch which was localized in the brain stem, in the caudal part of the pons.

In case 1 a slight perceptive hearing loss, without recruitment was found, and speech audiometry disclosed a pronounced loss of discrimination in the left ear indicating a retrocochlear lesion, Békésy audiometry with constant or interrupted tone did not show any tone decay except during simultaneous masking of the opposite ear. Blegvad & Therkildsen (1966) have found that in some patients with retro-cochlear lesions, Békésy audiometry produced a fatigue reaction only when the opposite ear was masked. This peculiar phenomenon may have some relationship to the efferent fibres to the cochlea (Blegvad, 1967).

Lesions of the branches of the trigeminal nerve, paresis of the facial nerve, and lack of vestibular function on the left side were likewise indicative of a process in the left posterior cranial fossa.

The acoustic reflex of the stapedius muscle could not be elicited on either the left or the right side during stimulation of the opposite ear. Absence of the reflex on the left side may be due to the paralysis of the left facial nerve. This is also in agreement with the lack of a homolateral reflex in this ear in spite of a nearly normal hearing level.

On the right side, a clear reflex could be demonstrated by homolateral stimulation. The results of the stapedius-muscle-reflex examination can only be interpreted as symptoms of a lesion cutting the reflex arch between the cochlear nuclei on the right side and the facial nucleus on the left side, while the homolateral reflex arch from the right cochlear nuclei to the facial nucleus on the same side remains intact (Fig. 1). Surgery disclosed a malignant tumour invading the brain stem in the pons.

In Case 2, hearing was normal. Békésy audiometry was not performed, but a continuous threshold recording test (Sørensen, 1962) showed a considerable tone decay on the left side, both with and without masking of the contralateral ear. On the right side, there was only a very slight tone decay unchanged by contralateral masking.

The caloric-test response was peculiar in spite of the equal durations of the responses. The nystagmus to the left did not appear, only a persistent deviation of the eyes to the right occurred, indicating a lesion in the brain stem reticular formation (Ethelberg & Vaernet, 1958).

In spite of normal hearing and no facial paralysis, stapedius muscle reflexes were absent in both ears during stimulation of the contralateral ear. Only homolateral stimulation resulted in normal reflexes. The most likely explanation of these indications must be an interruption of the reflex arch in the brain stem, involving the reflex arch from the cochlear nuclei to the facial nuclei on the opposite side (Fig. 1).

In these two cases, abnormalities in the stapedius reflex arch have been demonstrated. These abnormalities could not be detected by means of an ordinary impedance-measuring apparatus having facilities for contralateral stimulation alone. A device capable of homo-

lateral stimulation has been required. We wish to emphasize the value of such a device, as it enables us to contribute significantly to the diagnosis and localization of brain-stem affections, and may be of value in further investigations of the stapedius reflex arch.

ACKNOWLEDGMENT

We wish to express our thanks to E. B Neergaard M.Sc. for valuable help.

ZUSAMMENFASSUNG

Die Untersuchungen des Stapedius-Muskelreflexes sind and 2 Patienten vorgenommen worden. Die Reflexe wurden mit einer Impedanz-Apparatur gemessen, die nicht nur kontralateral, sondern auch homolateral stimulieren kann. An keinem der beiden Patienten konnte die kontralaterale Stimulation einen Reflex bewirken, während die homolaterale Stimulation einen normalen Reflex bewirken konnte. Diese Ergebnisse lassen sich nur wegen eines Abbruchs des Reflexbogens zwischen den cochleären Kernen der einen Seite und den Facialis Kernen der anderen Seite erklären. Ein Apparatur wie die oben erwähnte ist von grossem Wert bei der Diagnose und der Lokalisation der Hirnstamm Affektionen und bei weiteren Untersuchungen über den Reflexbogen des Stapediusmuskels.

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THE FREQUENCY INCREMENT SENSITIVITY TEST

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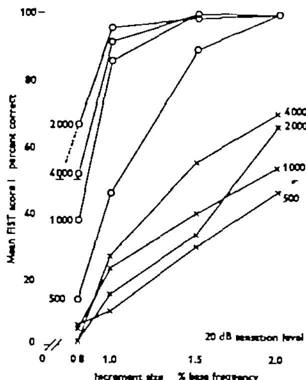
Abstract. Eleven normal-hearing subjects and 11 subjects ith cochlear hearing loss were tested with Frequency Increment Sensitivity Test (FIST). The FIST is an audiometric test using incremental frequency variations in a presentation and scoring method analogous to the SISI. A range of frequency increment sizes and range of sensation levels were employed. The mean score differences between the tw groups were highly significant ($P < 0.001$) at 20 dB sensation level with an increment size of 1.5% for 500 Hz and with an increment size of 1.0% for 1 000, 000, and 4 000 Hz. The FIST ppears to differentiate between normal-hearing subjects and those with cochlear hearing loss.

There are a number of auditory measures available to the audiologist when he begins a diagnostic sequence to determine site of lesion. Among these measures, the use of a test called Short Increment Sensitivity Index (SISI) has become very common in clinical diagnostic evaluations, while the use of a test called Difference Limen for Frequency (DLF) appears to have become much less common. The test under development in this study is an applica tion of the technique of the SISI to DLF The resulting test, called Frequency Increment Sensitivity Test (FIST), is intended to be a prac tical clinical test which may prove of value in differential diagnosis.

The clinical use of Difference Limen for Intensity (DLI) and Difference Limen for Fre quency (DIF) had a fairly parallel beginning in the late 1940s and 1950s, Lüscher & Zwislocki (1949), Denes & Naunton (1950), Jerger (1952) Meurman (1954) Filling (1957), Butler & Albrite (1957) In these early stages of development, the two procedures seemed to show an equal potential for aiding in the diagnosis of hearing disorders. The reasons why DLI was more readily accepted are not completely clear It is probable that the difference in predictability of DLI and DLF results from studies of recruitment as shown by direct measurement techniques, had a large measure of influence Another factor may have been the availability of the necessary equipment. Adapters used in DLI were easier to construct and less expensive than those required for DLF

The study of Ross et al. (1965) used a two-tone type DL measure in which both the DLF and DLI were obtained. Several other auditory measures were also made in an attempt to establish a measure that would be useful in predicting the speech discrimination score in quiet or in noise The results of the study re vealed a highly significant difference ($P < 0.001$) between the mean DLF scores of their normal-hearing group and their hearing-impaired group at all test frequencies The DLI results showed mean score differences which were significant ($P < 0.05$) only at 2 000 Hz, while the other test frequencies were not significant.

In recent years the SISI form of DLI (Jerger et al., 1959) has almost entirely supplanted all previous forms of DLI for clinical purposes, and has come to be very widely relied upon to aid in identifying the site of the lesion. The SISI



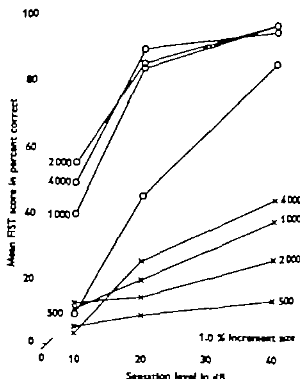


Fig 1 Mean FIST scores as function of increment size and of sensation level for base frequencies of 500 through 4 000 Hz. O—O normal-hearing group (= 11) — cochlear-hearing-loss group (n = 11).

jects scored greater than zero, one at 25% correct and one at 90% correct. For the 1.5% increment for 500 Hz, there were two 100% correct scores and three scores between 25 and 80% correct. At each of the three higher frequencies 1 subject achieved a high score and one or two achieved a mid score for the 1 0% increment at 20 dB SL, but only 1 subject was represented by more than one of these high scores. For the 2.0% increment, several 100% correct scores were noted for all frequencies, but at least one zero score remained.

The graph of the results of the FIST versus the increment size (Fig. 1) presents slowly rising curves for the cochlear-damaged subjects. The curves begin near zero at the 0.8% increment for all frequencies and rise finally to about 50% correct for the two lower frequencies and to about 70% correct at the two higher frequencies. The greatest spread between the curves of the normal-hearing subjects and the cochlear-damaged subjects is obtained for the 1.5% increment at 500 Hz and for the 1 0% increment for 1 000 2 000 and 4 000 Hz.

The effect of sensation level

In Fig. 1 the mean FIST scores of the two groups resulting from a constant 1 0% increment is shown as a function of the sensation levels of 10 20, and 40 dB Here the mean scores of the normal-hearing subjects tend to group very closely for the three upper frequencies, while the scores for 500 Hz are separated at the lower levels, but nearly join the grouping at the highest level. The rate of improvement in the score with increasing sensation level is rapid between 10 and 20 dB sensation level but slow between 20 and 40 dB sensation level for the upper three frequencies. The scores improvement at 500 Hz is again different from the rest, increasing at nearly the same rapid rate throughout.

The cochlear-damaged group continued to give low scores throughout. The graph of their results shows a moderate rate of score improve-

ment with increasing sensation level. But the improvement rate was somewhat different for each frequency such that the scores tended to spread among frequencies, rather than condense as in the normal-hearing group The greatest difference between the mean scores of the normal-hearing and cochlear-damaged groups appears at 20 dB sensation level for 1 000 2 000 and 4 000 Hz, and at 40 dB sensation level for 500 Hz.

DISCUSSION

The results of the use of the FIST on a group of normal hearing subjects and on a group with cochlear lesions indicate that a significant difference exists between the mean scores. The four objectives were realized. First, the adapter developed for the FIST seems practical for clinical use. Second, the increment size that resulted in significant differences at all frequencies used, was the 1.5% increment. However the highest *F* ratio occurred for the 1.0% increment for 1 000 2 000, and 4 000 Hz and for the 1.5% increment for 500 Hz. Third, the sensation level of 20 dB for 1.0% increment yielded highly significant differences at all three upper frequencies, indicating that 20 dB would be practical as a standard test condition. Fourth, significance was achieved ($P <$ 0.001) from the mean FIST scores for several of the test variations on the limited samples studied.

Fig. 2 is a representation of the mean scores obtained for the selected test variations recommended above, which yielded the highest *F* ratios, at 20 dB sensation level. Using the standard deviations obtained and drawing a vertical line from the mean score of each group mean at each frequency we see that one standard deviation from each group mean does not overlap It is desirable to apply two standard deviations to the recommended test value mean scores, in order to establish a limiting probable score for categorization purposes. Taking a very cautious view and picking a number that is a multiple of 10 one can infer that any score

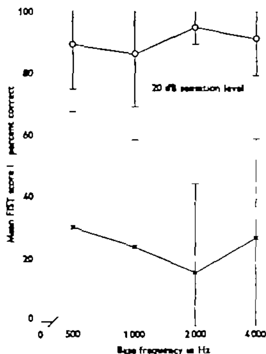


Fig. 2. Mean FIST scores for each frequency with one standard deviation shown by vertical bars. Test parameters used were: 1.5% for 500 Hz, 1.0 for 1 000 2 000, and 4 000 Hz. ○—○ normal, — cochlear $n=11$

less than 40% correct should be considered as coming from a damaged or pathological cochlea. The raw scores from this sample seem to bear this out, since not one score under the recommended test values fell below this level In fact a score of 45% correct occurs only twice among the scores of the eleven normal-hearing subjects.

Going the opposite direction to establish an upper score above which the cochlear-damaged subjects would not likely score is a very different case. One may say that any score greater than 80% correct *most likely* comes from a normal cochlea, but it would take a score of 100% or high scores at several frequencies, to make a more definitive statement.

Fig. 3 was constructed to provide a comparison with previous DLF research such as that of Parker et al. (1968) The DLF values were graphically determined from the 50% correct points of Fig. 1

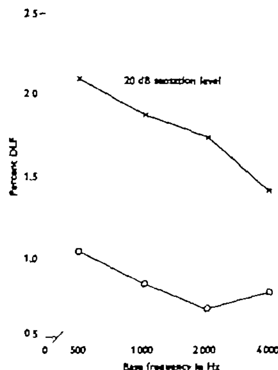


Fig 3 Percent DLF as extracted from 50% correct on mean FIST scores as a function of increment size (see Fig. 1). O—O normal-hearing group — cochlear loss group, *n* = 11

The subjective reaction to the FIST illustrates an important finding for its clinical use. The finding is the equivalence of the instructions for SISI and the FIST so that a single set of instructions is sufficient for both tests. It was also noted that many of the subjects did not distinguish the intensity increments from the frequency increments. Since the SISI immediately preceded the first FIST it was a simple matter to simply turn the SISI unit off and turn the FIST unit on, the base tone remaining continuous. With appropriate SISI and FIST increment choices many of the subjects responses continued to be consistent, without any apparent awareness that the stimulus had changed. This may permit the use of SISI and FIST increments alternately in short blocks to serve as mutual foil increments. A most unusual subjective report raises some doubts as to the nature of the percieved increments, but does not change the outcome of the test. The report came from a subject with known Menière's involvement, who when he did respond appropriately said that the tone appeared to go *off* at the increment.

CONCLUSIONS

This study has shown that the FIST is practical, that it can differentiate between normal and damaged cochleae, that certain increment sizes seem best, and that the 20 dB sensation level is usable However there are several uncertainties that have become apparent as result of this study With one exception, the subjects who comprised the cochlear-damaged group were not of medically confirmed etiology We have no knowledge of results in cases of VIII nerve lesion. Central disorders have not been explored with this test, nor even cases of conductive deafness. It is likely that contralateral masking may have an effect on the FIST score, but masking was not used in this study In spite of these uncertainties the FIST seems to have potential for aiding in differential diagnosis, but there is much yet to be known before the FIST is ready for routine clinical use.

RECOMMENDATIONS

It is recommended that the FIST be given further study with known etiology types, masking, and results correlated with other current differential diagnostic procedures.

It is recommended that for differential diagnostic purposes, the sensation level of 20 dB and the frequency increment sizes of 1.5% for 500 Hz, and 1.0% for 1 000, 2 000 and 4 000 Hz, be employed.

It is recommended that for the present the scores of 80% correct and above be considered as "Negative FIST" indicating normal cochlear function, and that scores of 40% correct and below be considered as "Positive FIST" indicating cochlear damage and that 40 to 80% correct be considered as "Questionable FIST"

SUMMARY

An audiometric test was developed which applied the test methodology of the SISI to a form of difference limen for frequency The new test, called Frequency Increment Sensitivity Test (FIST), was facilitated by a minor modification of an ordinary Beltone warble tone adapter Calibration of the modified adapter was accomplished by means of an oscilloscope, a second audio oscillator and a frequency counter

Eleven normal-hearing subjects and 11 subjects with cochlear-hearing-loss were administered eight variations of the FIST at each of four frequencies. The test variations consisted first of four different increment sizes, 0 8 1 0, 1.5 and 2.0% of the base frequency all at a constant 20 dB sensation level. Secondly three sensation levels, 10 20 and 40 dB were used, all with the 1 0% increment size. Finally a "catch all" combination of 2.0% increment with 40 dB sensation level was employed. The base frequencies used were 500 1 000, 2 000 and 4 000 Hz. An analysis of variance was performed on the mean scores of the two groups at each test variation for each frequency

The results of the testing yielded percent correct scores which, when plotted graphically appeared similar to the typical psychometric function ogive. The curves of the mean scores of the cochlear-hearing-loss group were lower and had a smaller slope than those of the normal-hearing group The differences between the mean scores of the normal listeners and listeners with cochlear hearing loss were shown to be highly significant ($P < 0\,001$) by analysis of variance for several of the test variations. The highest F ratio occurred when the sensation level of 20 dB was used with increment sizes of 1.5% for 500 Hz and of 1.0% for 1 000, 2 000 and 4 000 Hz. The null hypothesis of no clinically significant differences between the mean scores of the two groups was rejected.

The final conclusion was that the FIST had been shown to be effective with the limited samples studied, but that before routine clinical use is recommended, the respones of other known etiology types and the effect of masking must be known.

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ZUSAMMENFASSUNG

An elf normalhörenden Subjekten und elf Subjekten mit cochlearem Horverlust wurden der Frequency Increment Sensitivity Test (FIST) erprobt. Der FIST ist ein audiometrischer Test, der mit seinen zunehmenden Frequenzabweichungen in der Darstellung und Markierung dem SISI Test in vielem ähnlich sieht. Es wurde eine Skala für die Frequenzgrössen und die Empfindungsstufen angew ndt. Die durchschnittlichen (Marken-) grössendifferenzen zwischen den beiden Gruppen waren sehr bedeutungsvoll: (P < 0,001) bei 20 dB Empfindungsstuf mit einer Zunahme von 1,5 Prozent für 500 Hz und mit einer Zunahme von 1,0 Prozent für 1 000, 2 000 und 4 000 Hz Der FIST scheint den Unterschied zwischen normalhören den Subjekten und jenen mit cochlearem Horverlust darzustellen.

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STUDIES ON PROTEINS OF NASAL MUCOUS MEMBRANE SECRETION IN FIBRINOLYTIC ASPECT

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Received October 3 1969

Abstract. The secretion of the nasal mucous membrane was studied in healthy subjects and those suffering from allergic rhinitis, the so-called "third" fraction occurring in both groups was found to be lysozyme contaminated by the proteolytic enzyme activator.

The present paper is a continuation of our studies on fibrinolytic action of normal mucous membrane secretion of the nose. As already stated, the action is coupled with the protein the mobility of which is consistent with that of alfa$_2$, beta$_1$ globulins. However further studies with monovalent sera that precipitate protein from this group failed to give positive results, (Bednarski et al., 1969). In this connection, the present studies are mainly concerned with detailed characteristics of the protein fraction referred to as "third" fraction.

We have started from the view expressed by Remington (Remington et al., 1964) that one of non-identified proteins in the electrophorogram of nasal mucous membrane secretions might be represented by the lysozyme-α protein migrating to the cathode.

MATERIAL AND METHODS

The secretion of the nasal mucous membrane was sampled—after preliminary augmentation of the secretory function—by cooling the air inspired through the nose. In this way specimens were obtained from 10 children at the age of 8–14 years with established typical allergic rhinitis and from 10 normal persons without history of diseases of the upper respiratory tract for the last 6 months. Moreover tears from the conjunctival sac were taken in both groups.

TECHNIQUE

The whole of the specimen from one subject (both tears and nasal secretion) was divided into two groups: one of them was studied immunoelectrophoretically, the other being subjected to agar electrophoresis.

The agar was then cut into strips, 2 mm wide (numbered in the anode–cathode direction) and placed on *Micrococcus lyzodeikticus* culture. The result was read after 18 hours of storage at room temperature (+20 C). Moreover the experiment was checked with pure lysozyme (from hen egg) previously subjected to agar electrophoresis. By such a procedure it was possible to obtain a correlation existing between the lysis of the bacterial strain and the "third" fraction in the mucous membrane secretion of the nose. The comparison of immunophorograms also permitted the detection of eventual similarity between the precipitation arches of the "third" fraction occurring in the nasal secretion and those of protein fractions in the tears.

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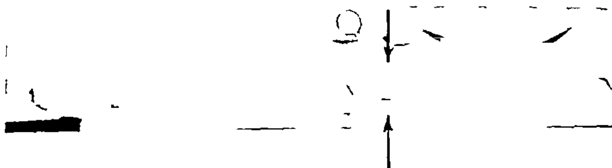


Fig. 1 Immunophorograms of nasal secretion and tears made simultaneously: arrows indicate "third" fraction of the nasal secretion and protein fraction of tears.

RESULTS

The precipitation arches of the "third" fraction in the nasal secretion and those from the lacrimal protein fraction were found to be identical as regards their appearance and distribution.

The secretion of the nasal mucous membrane, tear and pure lysozyme, when spread on agar strips with *Micrococcus lyzodeicticus* showed bacterial lysis in the area corresponding with the precipitation arch of the "third" fraction. The bacterial lysis of the lowest degree was produced by specimen contained in the tear.

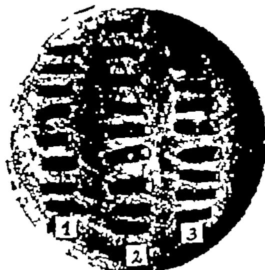


Fig. 2 Agar strips with electrophoretically separated specimen placed on *M. lysodeicticus* strain: Tear, 2 nasal secretion, 3 pure lysozyme. The most conspicuous bacterial lysis took place in group 3, the slightest one, in group 1. The lytic action of pure lysozyme was confined to bacterial strain, the fibrinogen being unaffected in this respect.

The above data, i.e. the identify of the precipitation arches in the phorogram of the tear and nasal secretion, as well as the presence of bacteriolytic action against *M. lyzodeicticus* in this area would justify the assumption that the "third" fraction is the lysozyme (muramidase).

This finding is by no means a revelation as regards the demonstration of the lysozyme in the nasal secretion since the phenomenon has been known from the studies by Fleming (1922).

It is, however, to be stressed that the detection and recognition of the lysozyme by means of immunophorogram is rather puzzling, as it has been reported that the lysozyme has poor antigenic properties and the precipitating serum is difficult to obtain (Ślopek, 1963, Josephson & Lockwood, 1964, Bonvida & Sapse 1967). On the other hand, our experiments resulting in recognition of the "third" protein fraction with fibrinolytic activity as being the lysozyme poses a new problem, which, to our knowledge, has not been tackled in the available literature.

Fibrinolytic aspects of the lysozyme present in the secretion of the nasal mucous membrane were envisaged from the theoretical point of view assuming that

1. it might possess a fibrinolytic effect,



Fig. 3. A fibrin plate with the dropping sites of
1. nasal secretion,
2. tears,
3. pure lysozyme. Fibrinolysis is visible in group 1 but absent from group 3

which seems rather doubtful, as suggested by our studies and those of other investigators (Fleming, 1922 Zabłocki, 1959 Slopek, 1963 Josephson & Lockwood, 1964 Bonvida & Sapse 1967) or

2. It is contaminated by proteolytic enzyme.

Ruling out the first point as being highly improbable, we are going to discuss the second one postulating its contammation. In this system, another possibility is to be considered, namely it must be remembered that the fibrin plates obtained are not clean, being contaminated by plasminogen that, in spite of a number of procedures used (heating, among others), could not be entirely eliminated without destruction of fibrin structure (cit. after Neurath, 1965) In this connection, not only the proteolytic enzyme but also the enzyme's activator might be incriminated as the contaminating factor: it would activate the plasminogen contained in fibrin plate and the latter after conversion into plasmin, produces fibrinolysis. Thus, the action of the activator might have been misinterpreted as being direct effect of the enzyme.

To check both possibilities, i.e. eventual a tion of the activator in the secretion, as m ated by plasminogen or exerted directly or brin in the case of existence of the enzyme, t following experiments had to be m.

The secretion of the nasal mucous m brane and pure lysozyme were proces e described in the previous paper additional aminations being concerned with their pr ase activity against casein

The studies were made with Unicam e trophorometer Sp.500 of English production wave length of 280 mm, pH 8.0 Results. The nasal secretion or pure lysozyme failed to produce protease activity against casein

This would prompt an assumption that the lysozyme present in the normal nasal mucous membrane and in allergic rhinitis is contaminated by the proteolytic enzyme activator. A similar phenomenon has been described in the available literature.

Other authors (Ali 1965 Neurath, 1965) stress that little is known about tissue activators of proteolytic enzymes, hypotheses being advanced that they may occur in two forms: proactivator and activator (cit. after W. Wołosowicz et al., 1969) The above results are somewhat at variance with those obtained by Sasaki (1968) who could demonstrate in the nasal mucous membrane the presence of unspecific fibrinolytic enzyme. These divergences might be due to the modified technique used by us that consisted in eliminating, to certain degree the action of activated plasminogen in the fibrin plate.

In the light of the present studies it may therefore be concluded that the action of proteolytic enzymes in the secretion from the nasal mucous membrane in healthy and allergic rhinitis, as well as in the nasal mucous membrane itself is still an open problem.

ACKNOWLEDGMENT

We wish to express our indebtedness to Professor Wiktor Jankowski, Director of the Oto-Rhino-Laryngologic Clinic in Wrocław for his valuable suggestions in the course of our work.

ZUSAMMENFASSUNG

Die Nasenschleimhautsekretion wurde bei 10 Gesunden und 10 Kranken mit allergischer Rhinitis untersucht. Die sogenannte „dritte in beiden Gruppen vorkommende Fraktion wurde identifiziert als ein Lysozym verunreinigt mit proteolytischem Enzymaktivator Die Verunreinigung wurde auch im Tränenenzym nicht aber im reinen Hühnereilysozym nachgewiesen.

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SPONTANEOUS CEREBROSPINAL FLUID RHINORRHOEA

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(Received March 31 1970)

Abstract The author reports 6 cases of spontaneous cerebrospinal fluid rhinorrhoea operated on at the Department of Otolaryngology Helsinki University Central Hospital, in 1965-9 The operations were carried out by the approach as used for external operations on ethmoidal sinuses. The fistula was closed with bone tissue, fascial and muscular grafts. In only 2 of these cases could the fistula be closed by a single operation. One of the patients needed three operations and 3 patients, two. Aplasia of the cribriform plates and the overlying meninges could be seen in all the cases. There were no fila olfactoria in the defective area of the cribriform plate, and in 2 cases a small dural prolapse with a fistula was seen in the bone defect.

Galen believed that a fluid, excreted from various parts of the brain and stored in the brain ventricles, escaped through the nose via the ethmoids (Kassel, 1912) Otto (1830) reported that holes could be found in the ethmoid bone and that these holes allowed water to pass out from the cerebral cavity through the nose

Cerebrospinal fluid rhinorrhoea (CFR), according to Cairns (1937), can be divided into the following groups.

1 Acute traumatic
2 Delayed post-traumatic
3 Following nasal surgery
4 Spontaneous

The CFR arising from traumatic causes is discussed by the present author in another article in this periodical (Grahne, 1970)

Spontaneous CFR is very uncommon. Coleman & Troland (1947) were prepared to accept only 1 case reported by St. Clair Thomson in 1899 and 12 others culled from the literature, as fulfilling their criteria of spontaneous CFR. Coleman & Troland (1946) themselves reported 3 further cases Single cases have been added by Cloward & Cunningham (1947) Dohlman (1948) Kleinfeld et al. (1950) Kalm (1951), Oldberg (1954) Berryman (1955) Sen Gupta (1956) Troland (1960) Bracewell & Carter (1963) Riser et al. (1964), Nussey (1966) Double records have been published by Love & Gay (1947) Rand (1953) and O'Connell (1964) The terms spontaneous, idiopathic and primary CFR have been used to describe these cases.

Coleman & Troland (1947) point out that the diagnosis of spontaneous CFR is very loosely used in the literature They consider that many cases of traumatic CFR are included in the descriptions of the spontaneous form. McDonald (1945) emphasizes that many cases can result from old injuries to which the patient apparently attached no significance. It is well known that CFR may occur long after the acute trauma. The present author has reported on a case of CFR occurring 15 years after the trauma (Grahne, 1970). Locke (1926) and Gros et al. (1968) class all cases of spontaneous CFR as tumour suspect and therefore insist on examinations to rule out the possibility of an intracranial tumour Locke (1926) points out that all tumours or conditions which lead to increased intracranial pressure after the

closure of the fontanelles and suture lines may also lead to CFR. When the intracranial pressure increases the cerebrospinal fluid (CF) can find its way out through an anatomically fragile portion of the skull base Sam & Kramer (1940) reported on a woman with a 3-year history of severe headaches relieved by the nasal leakage of large amounts of clear fluid. She was admitted to hospital in a state of fulminant meningitis. Roentgenological examination revealed a large pituitary fossa with complete destruction of the sellar floor The patient died after a short period, and autopsy revealed a large pituitary adenoma. In addition however there was a cranionasal fistula in the cribriform plate just lateral to the crista galli. Smith & Walter (1931) described a case with copius CFR of a 2 year duration. Operation revealed a cystic lesion of the pituitary eroding the floor of the sella. After the cyst had been removed without any attempt being made either to search for or to repair fistulas, the CFR ceased and did not recur There are records according to which a cranionasal fistula, if developing in connection with an intracranial tumour almost invariably communicates through the cribriform plate, remote from the tumour (Gotham et al., 1965). It is also known that intracranial tumours which erode the skull floor very seldom roduce CFR although CFR has been de-bed in connection with osteoma of the fron-al sinus (Ommaya, 1964) It is evident, however that CFR in connection with tumours can not be classified as the so-called spontaneous form of CFR, but it is extremely important to examine the patient very carefully with a view to the possible occurrence of a tumour

Many authors consider that raised intracranial pressure, even of a transient nature, is the basic factor in the onset of a spontaneous CFR. Locke (1926) assumed that there are three possibilities by which the CF could escape if under increased pressure:

1 Rupture of the arachnoid sleeves passing through the cribriform plate together with the olfactory nerve filaments.

2. Rupture of a congenital encephalocele from the meninges or frontal horn of a lateral ventricle through the cribriform plate.

3 Persistence of the embryonic olfactory tract ventricular lumen.

Today it is believed that the most probable pathway of the leakage in cases of spontaneous CFR is along the olfactory nerves. Each olfactory nerve consists of approximately 20 separate filaments which are axons of olfactory cells in the mucous membrane of the nose. These filaments pass from the mucous membrane, through the cribriform plate, to the olfactory bulb They are held together in bundles by extensions of three layers of meninges. The dura joins the periosteum while the pia mater fuses into the neurolemma of the nerves. The arachnoid does not usually extend through the cribriform plate, though when it does the extension is very short. It is apparently just this thin arachnoid and the mucous membrane which, for one reason or another are perforated and occasion the CFR. Usually no increased intracranial pressure can be noted. But it should be remembered that the cranionasal fistula functions as a safety valve so that the pressure cannot rise. This safety valve system can also be observed when CFR occurs in connection with brain tumours. Ommaya (1964) mentions that, in cases associated with aqueduct stenosis of the fourth ventricular tumours blocking the ventricular outflow the flow is often so profuse that the blockage and tumour do not produce the usual clinical evidence of their presence. It is assumed that in cases where a cranionasal fistula develops, there is a congenital defect in the cribriform plate which permits extension of an envelope of arachnoid along an olfactory nerve fibre. Johnston (1976) believes that fila olfactoria are congenitally absent in these cases and consequently CF can more easily open a pathway to the nose through the holes in the cribriform plate

The second alternative in Locke's (1926) list was rupture of a congenital encephalocele A meningocele can naturally also be involved. Reports in the medical literature of CFR following the removal of nasal polyps must be as

sumed to refer to cases in which an encephalocele or meningo-encephalocele had extended through the cribriform plate. In actual fact, it may be assumed that these meningo-encephaloceles had developed on the basis of the congenitally disturbed development discussed above in connection with Locke's assumption no 1

The third assumption Locke advanced was persistence of the embryonic olfactory tract ventricular lumen. This assumption has never been proved. An experimental study on animals, by Locke & Naffziger (1924) might perhaps be considered to support the assumption. Locke & Naffziger showed that colloidin masses injected under pressure into the subarachnoid space of dogs often escaped through the nose. They were able to prove that this leakage occurred through the cribriform plate along the sheath of the olfactory nerve filaments. This may possibly be taken to suggest that there is occasionally at least in the dog, some means of communication between endocranium and pharynx. On the other hand, however the pressure of injection may be assumed to have been so high that it perforated the arachnoid sleeves passing through the cribriform plate This also proves that, when it comes to resisting a raised intracranial pressure, the most fragile barrier between the brain and the nose exists in the region of the cribriform plate.

SYMPTOMS AND DIAGNOSIS

The most important symptom is naturally the outflow of a clear fluid through the nose The fluid has a salty taste. The position of the head usually influences the flow forward flexion, particularly making it greater CF leak usually begins in connection with an acute infection in the upper respiratory tract. Radiographs taken by ordinary techniques without contrast medium usually display normal conditions. Injections of radioactive isotopes into the lumbar canal (Crow et al., 1956 Di Chiro et al 1964 Sinanan et al., 1966) have been used to localize the cranionasal fistula. The injection of certain dyes into the lumbar canal and inspection of their escape from the nose may supply valuable information (Dohlman, 1948 Gotham et al., 1965 Kirchner & Proud, 1960) The fistula itself cannot be seen, however unless the test is made during the operation, otherwise only the site where the dye flows out into the nose is visible The present author finds (Grahne, 1970) that it is considerably simpler after a treatment with xylocain–adrenalin solution has contracted the nasal mucosa and conchae, to observe on inspection just where the clear CF runs into the nose The problem of differential diagnosis is often whether the fluid flows down medially or laterally of the concha media. If the flow is medial, and there is no history of trauma to the skull, the flow probably represents spontaneous CFR through the cribriform plate An examination must naturally be made to exclude the possibility of a tumour

Instillation of Pantopaque—a radio-opaque dye—into the nose and positioning the head so that the dye enters the anterior cranial fossa via the defect in the cribriform plate is a method successfully used by Teng et al (1963)

Anosmia or hyposmia have been encountered in a number of cases of spontaneous CFR, as described e.g. by Dohlman (1948) There was no instance of anosmia among the cases of traumatic CFR treated by the present author (Grahne, 1970)

THERAPY

Usually the cranionasal fistula in these cases has been closed via frontal craniotomy approach, using various tissues such as muscle fascia or artificial materials, e.g. methyl-methacrylate Even ear nose and throat surgeons have endeavoured to stop the flow by operation via a rhinology approach Dohlman [illegible] carried out a transnasal operation on a [illegible] with spontaneous CFR. He removed [illegible] form plate entirely and sutured the [illegible] sequently he loosened the mucosa

part of the nasal cavity and interposed a piece of muscle between the mucosa and the dura. The leakage ceased after this operation. Vrabec & Hallberg (1964) reported the successful re pair of a cranionasal fistula by the transnasal technique involving the use of a mucosal flap.

MATERIAL AND METHOD

The present series consisted of 6 patients, 4 women and 2 men, of ages ranging from 24 to 65 years, treated at the Department of Oto-laryngology Helsinki University Central Hospital.

The same method of operation was used in all cases apart from Case 1 The incision was made according to the approach used for ex ternal operations on ethmoidal sinuses. The upper ethmoidal cells were removed and the cribriform plate was exposed. The skull base was explored using the Zeiss operation microscope, with a 30 cm working distance. The enlargement was ×8 If a dural prolapse was found, bone tissue from the dorsum nasi was used to plug the bone defect and provide support for repositioning the prolapse. Then, and even if there was no dural prolapse, a piece of fascia lata was inserted under the cribriform plate, and a sizable piece of fascia and muscle was inserted under the fascia lata. The wound was closed without the use of nose packs. Antibiotics were administered postoperatively In a few cases the upper part of the nasal cavity was packed with gel foam

Case Report

Case 1

Male, 24 years. In 1962, in association with an acute common cold, clear fluid started flowing from the patient's left nasal cavity At first the flow was considered a symptom of allergic rhinitis. The CFR was extremely profuse, and the patient lost 300–400 ml CF per 24 hours. Operation was carried out on 4 September 1965 This operation, which was the first in the present series, was made through the septum of the nose. The upper part of the bony septum was removed, and from the mucoperiosteum in the roof of the nasal cavity the bone was detached far enough laterally to make the cerebrospinal fluid flow in between the septal layers. Spongiosa from the iliac crest was then inserted against the cribriform plate between the septal layers. As the operation was completed the flow of the fluid ceased. The incision was sutured and nasal cavities packed for a few days. The postoperative recovery was uneventful. There was no CFR for a week but subsequently it recommenced and increased in volume. The patient was discharged from hospital in the hope that cicatrization would gradually close the fistula. This hope failed, and the patient was re-admitted after 4 months. A second operation was carried out via an approach used for external operations on ethmoidal sinuses.

The operation consisted of left-side ethmoidectomy with exposure of the cribriform plate, in which an aperture 4 mm in diameter was detected. The cribriform plate was in this case unusually thick, for which reason the dura could not be inspected. The mucosa inside the bone defect was removed as thoroughly as possible. The whole ethmoidal sinus and the area underlying the cribriform plate were then filled with spongiosa taken from the iliac crest, while the nasal cavity was packed with gelfoam. Not even this operation succeeded in closing the fistula. CFR recommended again a week after the operation and continued until a third operation on 7 February 1967 This operation was carried out via the maxillary sinus. The cribriform plate was exposed and the fistula, on the same site as before, was closed with fascial and muscular graft. The area of the operation was packed with gelfoam and ribbon gauze. One week later there was again a very scant CFR but it ceased within a few months. Subsequently the patient has had no symptoms, and has felt no unsteadiness in his head. This was the only patient to be operated according to a method somewhat different from that described in Materials and Methods.

Case 2

Female, 46 years. In October 1966 in connec tion with a common cold, a flow of clear fluid started from the patient's left nasal cavity The patient had meningitis in February and again in April 1967

Operation was performed on 7 August 1967 An extensive defect was seen in the cribriform plate. The dural fistula could not be seen since no bone was removed. Some fascial and muscular tissue was inserted in the bone defect. After the operation the patient no longer had a heavy feeling in the head, and no more CF leakage was noted. On 14 September 1968 when the patient was engaged in heavy manual work which involved bending forward, a copious flow of fluid suddenly started from the left nasal cavity The leakage went on for 2 days and then ceased spontaneously The patient reported, however that her head felt heavy and uncertain as it had done before the operation. A second operation was carried out on 24 September 1968 As the defect in the anterior part of the cribriform plate was being explored, CFR started. In the bone defect there was a dural prolapse with a fistula. A peg of bone was inserted in the defect of the cribriform plate, inserting fascia and muscle underneath. Since this operation the patient has been symptom-free

Case 3

Female, 58 years. A "large nasal polyp" had been removed from the patient's left nasal cavity in 1930 The removal was immediately followed by profuse CFR, which gradually ceased, but began to reappear from time to time in the course of years. The patient had attacks of meningitis in 1942, 1959 1965 and 1967

Operation was carried out on 1 February 1968 A defect was noted in both the anterior and posterior parts of the cribriform plate. The dura seen in the anterior defect showed atrophic bluish patches. A dural prolapse bulged through the posterior defect, showing atrophic spots. The prolapse was repositioned A piece of fascia lata with some underlying muscle was inserted under the cribriform plate After the operation the patient was symptom-free and her head felt steady until, in connec tion with a common cold, the CFR recommenced in January 1969 At the same time the patient contracted meningococcic meningitis. On 13 March 1969 she was re-operated, and a cranionasal fistula was found in the dura of the bone defect in the posterior part of the cribrinoform plate. A new fascial and muscular graft was placed underneath the dura. The fluid leakage ceased but a month postopera tively again in connection with a common cold, the patient contracted a slight meningitis. Subsequently she has been symptom-free

Case 4

Female, 39 years. In the spring of 1964 in connection with a common cold, CFR began from the patient s left nasal cavity It ceased spontaneously after a year In April 1968 the CFR recommenced, and had continued ever since, profusely

Operation was carried out on 1 August 1968 A bone defect was noted in the anterior part of the right cribriform plate. The dura showed atrophic spots through which the arachnoid could be discerned. A piece of fascia lata with some underlying muscle was inserted under the cribriform plate. Since the operation the patient has been symptom-free. Her head now feels steady which it had not done since 1964 when the CFR occurred for the first time.

Case 5

Male, 40 years Watery discharge from the left nasal cavity had already occurred in the 1950s. The patient believed that he had allergic rhinitis owing to the dusty work he was doing as a carpenter In 1960 1962, and 1967 he had purulent pneumococcal meningitis. Pneumoencephalography performed at the Depart ment of Neurosurgery revealed that the left lateral ventricle was slightly enlarged owing to brain atrophy in the environment. Radiography of the skull gave no pathological findings.

Operation was carried out on 4 November 1968 The posterior part of the cribriform plate was missing. Here the dura showed several bluish, atrophic spots through which the ar achnoid could be discerned. A piece of fascia lata with some underlying muscle was inserted against the dura. The patient has been symptom-free since the operation. He reports that his head feels steady and very different from what it was before the operation.

Case 6

Female 65 years. In July 1966, in connection with a common cold the patient noticed a continuous drip of clear fluid from the left nasal cavity Pneumoencephalography per formed at the Department of Neurosurgery re vealed atrophy in the left hemisphere, espe cially in the frontal lobe, but no atrophic cavity was seen Operation on 17 September 1968 There was a bone defect in the anterior part of the cribriform plate with a dural prolapse The wall of the dural prolapse showed atrophic, bluish spots. At the tip of the prolapse there was a fistula with invaginated arachnoid, from which fluid leaked. In the posterior part of the cribriform plate there was also a bone defect with a smooth surface, where the dura had atrophic spots. The dural prolapse of the an- ır part was repositioned. A bone peg taken the dorsum nasi was inserted into the gap and embedded in a piece of fascia including some muscle A few days post peratively the CFR recommenced. Re-operaion was carried out on 12 December and two CF fistulas were noted in the dura of the posterior bone defect in the cribriform plate. Some fascia lata and muscle was placed underneath the cribriform plate. Twelve days after this operation a scanty flow of fluid began again. Recently however the CFR has been extremely scanty

DISCUSSION

None of the patients had any history of trauma to the skull They had all been examined preoperatively at the Department of Neurosurgery to exclude the possibility of brain tumours In 2 cases the pneumoencephalography suggested local "brain atrophy" It is possible that these radiological changes were signs of congenital aplasias The above description of the present series clearly demonstrates that, in connection with the so-called spontaneous form of CFR, there are congenital changes in the cribriform plate and its environment. The operative finding in all cases was congenital aplasia of the cribriform plate In 3 cases there was a bone defect only in the anterior part, in 1 case in the posterior part, and in 2 cases both in the anterior and posterior parts of the cribriform plate. The bone defects had smooth edges. No fila olfactoria passed through the dura in the areas of the bone defect. There were, however atrophic spots in the dura through which the arachnoid could be discerned by its bluish colour and it was always in one of these that the fistula was found. Most probably these atrophic spots are at the places where the fila olfactoria would normally pass through the dura. These patients had no fila olfactoria in the areas where the dura was exposed. Such atrophic areas, which underneath are lined by mucosa only make an extremely weak barrier between the endocranium and the nose. In an upper respiratory tract infection involving stasis, hyperaemia and mucosal infection, the bac teria can pass through this barrier and produce meningitis. The meningitis in its turn raises the intracranial pressure, and the result is a perforation of the barrier and CFR. Even a simple infection of the upper respiratory tract may produce a perforation of the barrier The stasis and hyperaemia caused by the infection may be complicated by a capillary thrombosis in the barrier An attack of coughing, or vigor ous nose-blowing, increase the intracranial pressure transiently and the result is perforation.

None of the patients had definite anosmia before the operation. A normal sense of smell or hyposmia on the side where the craniorasal fistula occurred were the preoperative findings. This seems to suggest the presence of a number of fila olfactoria outside the area of the

bone defect. Postoperatively the patients had anosmia on the operated side.

The operative findings concurred with those reported by Georgacopoulus (1951). He reported 4 cases of spontaneous CFR where no fila olfactoria could be found at the operation. *In a further case there was aplasia of the* cribriform plate. Georgacopoulus (1951) reported that he had found the same information in old operation records by Klein and Aubin, but published no detailed operative findings.

Testut (1911) described 2 autopsied cases with complete absence of fila olfactoria. He also reported on a further case in which the olfactory bulb and the olfactory nerves were completely missing.

Ramadier (1943) reported that osteo-meningeal dehiscence was often seen in these cases in the cribriform plate and the dura. It is considered that the pia and the arachnoid develop from the ectoderm while the dura and the bone tissue develop from the mesoderm. Consequently the cause of spontaneous CFR would depend on a developmental disorder in the mesoderm. Georgacopoulus (1951) pointed out that these congenital changes are very much like those seen in spina bifida where aplasia of the meninges and bone tissue is also involved.

In Cases 4 and 5 pneumoencephalography revealed a number of "atrophic" changes in the brain which may be considered attributable to congenital aplasia. Possiby (Case 6) they may also have developed as a result of disturbed circulation of the cerebrospinal fluid following attacks of meningitis.

In two of the present cases (Cases 3 and 6) there was a dural prolapse. In one (Case 3) a sizable meningo-encephalocele had previously been excised as a nasal polyp. The present operation revealed that this meningo-encephalocele had protruded through a defect in the cribriform plate. It may be assumed that the etiological mechanism of the encephalocele was roughly the same as that of a dural prolapse.



Fig. 1. Fistula in the cribriform plate indicated by the black arrow.

When these patients were radiographed, contrast medium (Pantopaque) was instilled into the nasal cavity with the patient's head tilted right back, according to Teng et al. (1963). In Cases 3 and 6 the fistula in the cribriform plate could be localized. In both of these cases there was a small dural prolapse, possibly inverted while the head was tilted so far back (Fig. 1). In the other cases the finding was inconclusive.

The operative closing by extracranial approach of the cranionasal fistulas, in the 6 cases now described, proved to be much more difficult than the treatment of the 13 cases of traumatic cranionasal fistula reported by the present author earlier in this periodical (Grahne, 1970). One patient (Case 1) had to have three operations, while 3 patients had two operations, and even then 1 of the three still has slight CFR from time to time. In only 2 cases could the fistula be closed in one opera-

tion. It must be pointed out, however that these operations with extracranial approach never produce any brain damage, and they involve no risk to the patient.

It is difficult to imagine that a cauterization of the environment of a cranionasal fistula, as proposed by de Almeida (1928) could possibly close a major fistula in the cribriform plate. Nor can one expect to reach any results with X-ray irradiation, a treatment suggested by Sgalitzer (1930) or with repeated lumbar punctures which were proposed by Singleton (1931) and practised by Frenzel (1960)

ZUSAMMENFASSUNG

Der Verf sser berichtet über sechs Fälle von spontanem Liquorabfluss aus der Nase, die in der Oto-laryngologischen Klinik der Universität Helsinki in den Jahren 1965–1969 operiert worden sind. Die Operation wurde nach einem Verfahren ausgeführt, das bei externen Operationen des ethmoidalen Sinus gebräuchlich ist. Die Fistel wurde mit Knochengewebe, Fascia lata und Muskel geschlossen. Nur in zwei Fällen konnte die Fistel mit einer einzigen Operation geschlossen werden, in drei Fällen musste der Patient zweimal operiert werden und in einem dreimal. Aplasie der Lamina cribrosa und der darüberliegenden Meningen konnte in allen Fällen konstatiert werden. Die Fila olfactoria fehlten im Bereich des Knochendefekts in der Lamina cribrosa. I zwei Fällen wurde auch ein kleiner Duraprolaps mit einer Fistel im Knochendefekt in der Lamina cribrosa gefunden.

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TRAUMATIC CRANIONASAL FISTULAS PERSISTENT CEREBROSPINAL FLUID RHINORRHOEA AND THEIR REPAIR WITH FRONTAL SINUS OSTEOPLASTY

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Abstract. The incidence of skull injuries has continuously increased in recent years and may be expected to go on increasing in the future Road accidents frequently produce frontobasal skull fractures. Cerebrospinal fluid rhinorrhoea is a serious complication in these frontobasal skull fractures. The condition is discussed in the first section of the paper It is pointed out that most of the cases of chronic cerebrospinal fluid rhinorrhoea show the cranionasal fistula in the posterior wall of the frontal sinus. The reasons for this are also discussed.

An extracranial operative method for their treatment is described. This method was used in 13 chronic cases of cerebrospinal fluid rhinorrhoea, and proved successful in all of them. The method involves frontal sinus osteoplasty through a curved incision from the back of the nose to the underside of the eyebrow with axillary incision up toward the forehead. The an[illegible]or wall of the frontal sinus is removed by drilling. [illegible] the mucosa of the frontal sinus is meticulously removed. The nasofrontal duct is hermetically sealed with a peg of bone taken from the iliac crest. A plate of spongiosa is placed across the cranionasal fistula, and the whole frontal sinus is filled with spongiosa. The anterior wall of the frontal sinus is reconstructed with mosaic plastics of cortical bone. The incision is closed without drainage and dressed with a compression bandage

The number of road accidents increases more and more drastically every year The victims of these accidents, especially those sitting in the front seat of the car often sustain a fracture of the anterior part of the skull. The hectic tempo of work in modern factories also produces skull injuries. Fractures of the vault of the skull usually include both tables of bone When the inner or the posterior vault of the frontal sinus is fractured the dura and the arachnoid are often ruptured and cerebrospinal fluid rhinorrhoea (CFR) occurs This must be considered a compound fracture exposing the cranial cavity and its contents to infection.

The acute injury

The first evidence of CFR associated with *fracture of the skull is the occurrence of a* watery or blood-tinged discharge from the nose The presence of CFR is not an indication for immediate repair of the fistula. In many cases repositioning and fixation of fractured facial bones must be undertaken almost immediately after the accident. This applies particularly to cases with large open wounds, since it is preferable for the operation to advance, so to say from the interior outward. The reposition and fixation of the fracture may result in a cessation of cerebrospinal fluid leak. This is especially true if the fracture is in the ethmoids. If the operation is not performed immediately the head of the bed must be elevated to an angle of approximately 45 degrees The patient should be restrained from blowing his nose Nasal packs, which may prevent adequate drainage and later occasion infection, should be avoided. Antibiotic therapy must be instituted at once Smoking is not permitted. Undue manipulation of the fractures should be avoided. In severe skull injuries damage may have been in-

flicted to important regions in the basal part of the brain, with associated severe lesions in the cerebral cortex. Treatment of the paranasal sinus fracture in these severe cases must sometimes be postponed or completely abandoned to give the patient the best chance of surviving the first, difficult post-traumatic period. CFR usually stops spontaneously some time after the trauma, although there are many cases in which it does not cease until a reduction and fixation of the paranasal sinus fracture has been performed (Boering & Beks, 1963 Dingman, 1964). In a number of cases there is no CFR even though the posterior wall of the frontal sinus is fractured, with a tear in the dura and arachnoid. Lewin (1954) and Escher (1969) found in these cases that a brain fungus may fill the large bony defect in the paranasal sinuses and thus prevent the escape of cerebrospinal fluid.

There are many reasons why CFR may fail to cease spontaneously. A fibrous barrier effectively closing the subarachnoidal space, is not always formed. The most important reason why such a barrier cannot develop especially in the frontal sinus, is that there is no bed, there being only the air cavity of the sinus, from which fibroblastic proliferation could grow. It is also possible that the sinus mucosa, which usually grows very fast, may close the defect. This barrier is easily ruptured. Adson (1941) assumed that a profuse cerebrospinal fluid leak through the fistula might prevent spontaneous closing. Inclusion of the dura and arachnoid between the fragments of the bone can also be the causative factor preventing the fistula from closing. Another factor to prevent healing of the fistula may be the enlargement of the fracture by the pulsating brain. Yet another reason is infection which may break through a newly formed barrier by means of the following mechanism. Haemorrhage from the fracture in the sinus wall fills the frontal sinus with blood. Some of the blood runs in a few days at the latest, through the nasofrontal duct to the nasal cavity. This irritates the patient who reacts by blowing his nose to remove the blood as a result, infective material is forced under pressure into the frontal sinus. The mucosa of the frontal sinus is thus infected and swells, blocking the nasofrontal duct. The inflammatory process in the sinus increases the intrasinus pressure, with the result that infected matter is forced into the intracranial cavity through the newly formed thin barrier.

Site of fistula in persistent CFR

Most of the cranionasal fistulas in frontobasal fractures are located, either totally or partly in the posterior wall of the frontal sinus (Teachenor 1927). If there is a persistent CFR the fistula is most frequently situated in the posterior wall of the frontal sinus and not in the cribriform plate. This concurs with Johnson & Dutt's (1941) observations on the anatomy of fractures in this region. They were able to demonstrate that even when the roof of the ethmoidal labyrinth was fractured the cribriform plate itself was usually intact. The fracture mostly runs on either side of, and posterior to the cribriform plate. The T-shaped mass of bone formed by crista galli and the cribriform plate itself is a tough reticulum of cords of dense bone. Thus the fracture lines tend to pass around the cribriform plate. If the fracture runs through the ethmoids along the T-shaped mass of bone it passes through several small air cells, each lined with mucosa. The CFR irritates the mucosa of these air cells. The mucosa becomes hypertrophic filling the whole air cavity. This means that the fistula is surrounded by a thick bed from which fibroplastic proliferation can grow to close the defect. The conditions are similar to those in the temporal bone. The relatively long cerebrospinal fluid (CF) fistulas in the temporal bone close, in most cases, spontaneously. The conditions are reversed, however if the fracture is located in the thin posterior wall of the frontal sinus, ad cent to a large air cavity. It is standabl that a large pro sin fistulas, which do must be located in sinuses. For the

tulas with CFR in the sphenoidal sinus have a poor prognosis as far as their healing is concerned. These fistulas are rare in the frontobasal skull injuries and have been successfully closed by extracranial surgery (Wersäll, 1969)

Symptoms and diagnosis

In cases of persistent CFR the discharge is clear and watery It may be continuous, or may be interrupted for a few hours before recommencing The discharge may appear as a drop from the nose or a backward flow into the nasopharynx, to be swallowed Usually it appears from only one nasal cavity producing a sensation of congestion of the nose. The discharge is often accentuated by manual work especially if leaning forward is involved. The daily amount of CF varies from case to case, from a few drops to ounces.

The patients often complain of headache a dull continuous headache increasing with manual work, which may be due to a low pressure from the steady loss of CF Another indication of this low pressure is vertigo experienced by these patients when the discharge increased for example when working in a forward leaning posture.

Quist Hanssen (1961) suggested the use of aper tape impregnated with specific enzymes reating glucose (Clinistix®) to demonstrate e presence of CF Gadeholt (1964) and Kirsch (1967) showed, however that a positive reaction can sometimes be obtained from nasal secretions even though no CF is present. But if the test is carried out strictly according to the manufacturers instructions the method is in any case helpful in the diagnosis of CF leakage.

A chemical analysis of the fluid, however provides more conclusive information.

Roentgenological verification of a suspected fracture in the region of the frontal sinus and the floor of the anterior cranial fossa is particularly difficult. This is due to the thin bone structure and complicated architecture so that fractures are not always revealed. Opacity of the frontal sinus or verification of a thickened mucosa may often be the only roentgenological signs of a fracture with cranionasal fistula. In some cases tomography may be helpful.

The association of CFR with air in the ventricular system or in the brain substance is termed pneumocephalus Chiari (1884) was the first to describe this condition in a case he autopsied. Luckett (1913) was the first to detect air by radiography in the ventricles, following a fracture of the skull. Pneumocephalus is a very rare finding. Coleman (1937) reported that he had seen the condition only four times over many years. He worked as neurosurgeon in a hospital where for example in 1935–36, 216 fractures of the skull were treated Lewin (1954) on the other hand, stated that of a total series of 308 patients with non-missile head injuries with fractures involving the para nasal sinuses, intracranial air was present in 24 Other diagnostic tests have also been employed, such as injection of the blue stain cystachrome (Dohlman 1948) fluorescein (Kirchner & Proud 1960· Briant & Snell, 1967) or indigocarmine (Gotham et al., 1965) into the lumbar theca or ventricle and observing its escape from the nose. Radioactive isotopes may be similarly employed (Crow et al 1956 DiChiro et al., 1964 Sinanan et al., 1966)

Frequency

There is no indication in the literature as to the percentage of cases with CFR which become chronic. In the pre-antibiotic era, such cases were extremely few since meningitis usually developed sooner or later and the patient usually died during the first attack. There are a number of reports on CFR in connection with acute head injuries Lewin (1954) for example, showed that of 1 000 consecutive cases of non missile head injuries severe enough to be admitted to hospital, 72 were found, on radiography or at autopsy to have a fracture involving the paranasal sinuses (7.2%) Of these 18 developed unequivocal CFR, nearly 2% of the total Calvert (1942) analysed 128 cases of paranasal sinus fractures, in which 21 (16%) had CFR. The percentages mentioned apply to CFR associated with acute trauma. The head injury may

have been relatively slight without a clinically demonstrable skull fracture. McDonald (1945) noted that some cases of so-called spontaneous or idiopathic CFR can result from old injuries to which the patient had apparently attached no significance.

Treatment

As indicated above, most cases CFR of traumatic origin cease spontaneously Dandy (1926), Teachenor (1927) Cairns (1937) and Coleman (1937) have advocated surgical repair of the meninges if CFR does not disappear within 4 to 6 days of the trauma. They used a transfrontal sinus approach in which the dura was exposed and repaired at the point of injury This surgical repair of the meninges, however proved to be very difficult technically and the mortality rate in the pre-antibiotic era was very high. Teachenor (1927) reported on two series of treated patients. In the first series, the operation was performed immediately after the trauma and the mortality rate was 37.5% In the second series, in which the operation was performed somewhat later the mortality rate was 87% Teachenor (1927) has also suggested removal of the posterior wall of the frontal sinus by craniotomy If, in these acute cases, there was any evidence of comminution with fragments of bone in the frontal lobes, an intracranial operation was carried out. Usually the neurosurgeons performed the operations via a craniotomy approach and preferred an intradural to an extradural approach.

Gotham et al. (1965) recommended that the conservative treatment of CFR should be maintained for up to 6 weeks after trauma The neurosurgeons have usually employed frontal craniotomy for the treatment of persistent cases of CFR. The defect was sealed with muscle (Cairns, 1937 Adson 1941 Herlin 1969) fascial grafts (Dandy 1926 Gurdjian & Webster 1953) and methacrylate (Thomas et al 1960 Gotham et al., 1965) Berendes (1957) used an extracranial method of bending up tongue-shaped flaps against the posterior wall of the frontal sinus. Escher (1969) later than Frenckner & Richtnér (1960) recommended operation at an early phase after the trauma. Escher (1969) had also tongue-shaped flaps or a free fascial graft to close the fistula. Aboulker et al. (1966) reported 15 cases of cranionasal fistulas treated via an extracranial approach

The present method

The writer described his method at the Scandinavian otolaryngological congress in Helsinki 1966 (Grahne 1967) The method has subsequently only been modified in regard to the incision and the reconstruction of the anterior wall of the frontal sinus.

A curved incision is made from the frontal process of the maxillary bone along the underside of the eyebrow with an auxiliary incision upward towards the forehead (Fig. 1) The incision passes through the periosteum and continues laterally just so far as to avoid cutting branches of the supraorbital nerve. The periosteum is freed from bone With an electric drill the surgeon gains access to the frontal sinus. The main part of the anterior wall of the frontal sinus is drilled off until the whole sinus can be viewed. In a number of cases the whole anterior wall was removed by the drill to reach all recesses of the sinus (Fig. 2) The mucosa is then carefully removed A small double curette is first used to remove the bulk of the mucosa. The last remnants are scrupulously removed with an electric drill. The mucosa must also be very carefully taken away from the upper opening of the nasofrontal duct. Any mucosa that has grown over the dura is also removed carefully The dural tear is not sutured Bon tissue is taken from the iliac crest, both cortical bone and spongiosa. A peg of bone is first formed, with one sharp end and with the longitudinal side consisting of cortical bone and the other of spongiosa. The peg of bone is forced into the nasofrontal duct which is thus securely plugged and sealed. A plate of spongiosa is placed across the dural tear and the whole frontal sinus is packed with spongiosa If the intersinus septum is intact only one of the frontal sinuses is filled If it

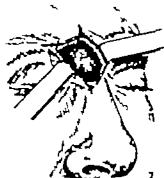
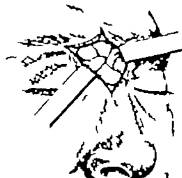




Fig 1 The incision.
Fig 2 The anterior wall of the frontal sinus drilled off.
Fig 3 The mosaic plastics of cortical bone

not intact, the other frontal sinus is also filled by a corresponding contralateral approach. Any air cell that may be present in the intersinus septum is opened and the mucosa removed When the whole frontal sinus is tightly packed with spongiosa, a mosaic of cortical bone is built to fit closely into the aperture in the anterior wall of the frontal sinus (Fig. 3) The periosteum and subcutaneous tissue are sutured in one layer and the skin in another No drain is used The wound is dressed with a compression bandage Antibiotic therapy is administered for 8 days postoperatively All operations in the thirteen cases described below were performed according to this technique der intubation anaesthesia. The cosmetic result of the operation was good (Fig. 4)

DISCUSSION

The extracranial method of operation described above has, among others, the advantage that it does no further damage to the brain which already been injured by the trauma Besides it is a minor operation compared with intracranial methods The method gave good results in all the cases treated. All the cranionasal fistulas in the frontal sinus could be closed by this method. In 2 cases (Cases 5 and 6—see Table I) with extensive fractures in the skull base an extracranial operation was also performed to close a fistula outside the frontal sinus.

The fistulas in the dura and arachnoid need not be sutured. The dura in the region of the posterior wall of the frontal sinuses is extremely thin and fragile, which makes suturing very difficult technically as pointed out by Grant (1923) and Herlin (1969) The dural tear closes itself if a bed is made from which fibroplastic proliferation with cicatrix formation can grow The fistula is hermetically sealed at operation by the bone peg packed into nasofrontal duct This bone peg also covers any fractures in the nasofrontal duct itself This was particularly important in the operations in those cases where the fracture was located in the upper part of the nasofrontal duct. The frontal sinus becomes ossified and, in radiograms, sometimes shows



Fig 4 Postoperative photograph.

a structure almost indistinguishable from that of the surrounding frontal bone.

To avoid cutting off branches of the supraorbital nerve, it is important that the incision on the underside of the eyebrow should not be too long laterally. For this reason a second incision is made in the form of a rising curve towards the forehead. If required, it is also possible to distend the operation wound by means of retractors. In this way the nerve branches remain intact. Nerve damage, if any caused by distension heals spontaneously.

In order to ensure good visual control inside the frontal sinus, the opening in the anterior wall must be made large enough. The method described will even permit the removal of the whole anterior wall by drilling. The electric drill has proved particularly suitable for removing the last remnants of mucosa. Particular care must be used in the removal of the mucosa from the upper opening of the nasofrontal duct, where it is especially liable to regenerate.

The craniosinus fistula is not always situated on the same side as the CF leak. This was seen at operation in Cases 3 and 4.

Radiological diagnosis of the fracture in the posterior wall of the frontal sinus is difficult, as pointed out earlier. The present author would like to emphasize the importance of suspecting a fracture in the event of radiological opacity in any of the frontal sinuses in CFR. The mucosa in the nasal cavity through which the CF leaks, has in all cases shown a certain degree of congestion. The mucosa is apparently irritated by the CF. Its sugar content is not compatible with the physiological properties of nasal mucosa. We saw the veracity of the reports in the literature stating that a handkerchief used by patients with CFR does not, after drying, show the stiff characteristics typical of mucous membrane secretions (Coleman & Troland, 1947. Lewin, 1954).

To ease the congestion of nasal mucosa, the present author packed the nasal cavities with cottonwool moistened with xylocain–adrenalin solution. In 10–15 min the cottonwool was removed, after which the nasal cavity was easy to inspect. The CF could be seen to run down into the nose laterally to the middle turbinate where the nasofrontal duct enters the nose.

The onset of CFR can sometimes be considerably delayed in relation to the trauma. Apparently the primary dura-arachnoid tear closes with atrophic scar which later in connection with an infection in the upper respiratory tract, gives way, permitting a CF leak. It is possible that in a number of cases a brain prolapse can plug up the fistula and then, later be resorbed. Another assumption might also be that the tear is closed only by frontal sinus mucosa forming a weak barrier which cannot last. The onset of CFR may coincide with the patient's first attempt to stand upright. This posture naturally makes a greater demand on the weak barrier which breaks under the strain.

Six of the 13 cases had a history of recurrent meningitis. Case 1 had had meningitis about 30 times. It is evident that meningitis no longer carries the high mortality of the pre antibiotic era, during which these patients mostly succumbed to the first attack. There is therefore a great need for operative therapy in these chronic cases today.

ZUSAMMENFASSUNG

Schädelfrakturen sind im Laufe der letzten Jahre ständig häufiger vorgekommen und werden künftig noch zunehmen. Autounfälle führen nicht selten zu frontobasalen Schädelfrakturen. Eine schwere Komplikation dieser frontobasalen Schädelfrakturen ist die cerebrospinale Liquorrhöe. Diese Komplikation wird im ersten Teil der Arbeit behandelt. Der Verfasser stellt hier fest, dass in den Fällen von Liquorrhoe, die chronisch geworden sind, die kranionasale Fistel meistens in der hinteren Stirnhöhlenwand liegt. Im zweiten Teil werden dann die Ursachen hierfür besprochen. Der Verfasser beschreibt eine extrakranielle Operationsmethode dieser Fälle. Er hat diese Methode mit gutem Resultat in dreizehn chronischen Fällen von Liquorrhöe angewandt. Die Methode zielt auf eine Osteoplastik des Sinus frontalis hin. Zunächst wird ein Bogenschnitt vom Nasenrücken zur Unterseite der einen Augenbraue geführt, danach ein Hilfsschnitt hinauf zur Stirn. Die vordere Stirnhöhlenwand wird weggebohrt und die Schleimhaut sorgfältig abgetragen. Der Ductus nasofrontalis wird hermetisch mit einem Knochenpflock aus der Crista ilica verschlossen. Auf die Fistelöffnung wird eine Spongiosaplatte gelegt, wonach der ganze Sinus frontalis mit Spon

Table I *Series of thirteen cases of traumatic cran[illegible]asal fistulas with persistent CFR*

Case No.	Sex, age	Time elapsed since original trauma	CFR preoperatively since	Notes	Operated upon in	Site of CF fistula in the frontal sinus	Recurrence of CFR postoperatively
1	♂ 9	3 years	3 years	30 (sic!) attacks of meningitis	1965	Posterior wall of the left sinus	No recurrence
2	♂ 36	9 months	9 months	One attack of meningitis	1965	Posterior wall of the right sinus	No recurrence
3	♂ 39	15 years	6 months	Daily periods of increasing headache transiently relieved by profuse CFR. No meningitis	1965	Lowest part of the posterior wall of the left sinus	No recurrence
4	♂ 41	18 months	3 months	Pneumocephalus. One attack of meningitis	1966	Lowest part of the posterior wall of the *right* sinus with CFR into the *left* nasofrontal duct.	No recurrence
5	♂ 21	15 years	15 years recurrent	Penetrating trauma. Six attacks of meningitis	1966	Lowest part of the posterior wall of the *left* sinus. Meningocerebral prolapse	Recurrence of CFR 3 years later with CFR from the *left* nasal cavity. Operated upon. Posterior part of the *right* lamina cribrosa missing, meningocerebral prolapse with CF fistula. Since operation no recurrence of CFR
6	♂ [illegible]	15 years	15 years continuously	Penetrating trauma. 5 attacks of meningitis	1966	Lowest part of the posterior wall of the left sinus	No recurrence, but 1967 attack of meningitis. Orbitotomy and duraplasty performed
7	♂ 54	27 years	16 years, continuously	Rifle bullet. 8 attacks of meningitis	1966	Lower posterior wall of the right sinus	No recurrence
8	♂ 1	12 years	8 years, continuously	Attempts to close the CF fistula by neurosurgeons 1959 and 1962. No meningitis	1967	Posterior wall of the left sinus. Meningocerebral prolapse	No recurrence
9	♂ 16	5 months	5 months	Pneumocephalus. No meningitis	1967	Posterior wall of the right sinus. Meningocerebral prolapse	No recurrence
10	♂ 56	4 months	4 months	Gun shot through the forehead. Pneumoencephalography and angiography revealed [illegible]	1967	Lower posterior wall of the left sinus	No recurrence

				[illegible] brain atrophy in the frontal lobes. No meningitis			
11	♀ 24	7 years	7 years continuously	No meningitis	1967	Posterior wall of the right sinus. Meningocerebral prolapse	No recurrence
1	♂ 24	3 years	1 year	Severe maxillofacial trauma. Daily attacks of increasing headache transiently relieved by profuse CFR. One attack of meningitis	1968	Lower posterior wall of the right sinus	No recurrence
13	♂ 16	1 month	1 month	No meningitis	1968	Lower posterior wall of the right sinus	No recurrence

sagewebe ausgefüllt wird. Die vordere Stirnhöhlenwand wird mit einem Mosaik aus Corticalisplatten rekonstruiert. Die Inzision wird ohne Drainage geschlossen und mit einem Kompressionsverband versehen.

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EXPERIMENTAL TRACHEAL RECONSTRUCTION WITH COMPOSITE GRAFT FROM NASAL SEPTUM

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Abstract. A composite graft from the nasal septum, consisting of cartilage with attached mucosa, was transplanted to window defect in the cervical trachea in 5 dogs. No respiratory symptom appeared during the observation time f 11 to 12 months. Four dogs had only slightly reduced tracheal dimensions in the reconstructed region, but one had a pronounced stenosis, which might be due to an anesthesiological complication. There were only remnants f the transplanted cartilage. The mucosa had principally ciliated columnar epithelium but the central part had often nonciliated cuboidal epithelium. Since the anterior part of the nasal septum in dogs has squamous epithelium it seems likely that transformation to columnar epithelium had occurred. Mucus transport over the central part of the grafted place was only observed in one dog. The possibility of utilizing the described method in man is discussed.

Tracheal defects which require reconstruction often entail great therapeutic problems. Defects which involve the tracheal cartilage have a tendency to give stenosis.

A short stenosis can be treated with resection and end-to-end anastomosis. Longer stenoses are usually treated with stents or dilatators of different kinds.

Many reports have been published about reconstructive procedures using various materials which substitute for the tracheal wall. These operations can be divided into those repairing defects of the whole circumference and those covering a window defect. In the first group may be mentioned the work by Ekeström (1958). He implanted Teflon rings connected by small bridges in the submucous tissue of the abdomen in dogs. A tube consisting of these Teflon rings and connective tissue was later transplanted to the resected trachea. Short plastic tubes were used as internal support at each anastomosis in a series of 25 dogs and 13 of these were living in good condition after 1 year. Ekeström & Carlens (1959) applied this technique with success in two patients. McCaughan (1968) found that silastic reinforced with Dacron was unacceptable as tracheal graft due to granulations and stenosis.

Window defects are present after excision of tracheal tumors and are also found in tracheal stenoses which have been cut longitudinally in order to interpose a graft (Gibson, 1967).

Various metals or synthetic materials such as stainless steel mesh (Bucher et al 1951, Edgerton & Zovickian, 1954, Keshishian et al., 1956), Ivalon (Björk & Rodriguez, 1958), Tantalum (Greenberg, 1960), Marlex (Beall et al., 1967) and Silicon-Dacron (McCaughan, 1968) have been used for tracheal reconstruction. Grafts of different tissues have also been used such as skin (Gebauer 1951, McComb 1967, Yasargil, 1967), fascia (Belsey 1950, Swift et al., 1952, Cahan, 1952), periosteum (Sato et al., 1957), costal cartilage (Sato et al 1957), bone (Pressman, 1953, Sato et al 1957), oral mucosa (Meyer 1963), urinary bladder mucosa (Rush & Cliffton, 1956), some

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Fig 3 The transected trachea in the dog with tracheal stenosis. The ends of the cut tracheal rings have fallen together

consisted of a membrane separating the ends of the cut tracheal rings (Fig. 5) Macroscopically there were almost no remnants of the cartilage in the graft The inner surface was covered by a mucous membrane The centre of the previous graft appeared as a scar and one had a small granulation in the centre of e scar

Film studies of the mucus transportation showed that the indian ink moved in caudal-cephal direction in the trachea passing around but not over the scar except in one dog. However there was no accumulation of mucus at the caudal border of the scar In the dog which had mucus flow over the scar this transportation always occurred obliquely from left to right.

Histological examination showed that the septal cartilage had almost disappeared and only small remnants of this cartilage were observed (Fig. 5) A membrane of connective tissue was present at the place of the graft. This membrane was in four of the dogs somewhat smaller than the original graft, while the dog with stenosis only had a very narrow membrane Serial sections of the membrane showed columnar epithelium in most parts. The epithelium was usually higher in the peripheral than in the central part of the scar where it was of cuboidal kind Squamous epithelium was observed only in a small area in the dog with a central granulation. Most columnar cells had cilia (Fig. 5) The cilia were less abundant and less regular arranged in the central part of the scar than in the peripheral The cuboidal cells in the centrum of the scar had usually no cilia (Fig. 5)

DISCUSSION

Transplantation of a composite graft from the nasal septum to a window defect in the trachea may theoretically have certain advantages over other grafts due to the similarities of the tissues in the tracheal wall and in the nasal septum. Transplantation in five dogs was not followed

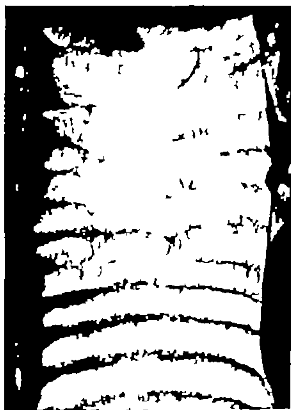


Fig 4 Trachea, opened posteriorly in one of the four dogs with only slight reduction of the tracheal size A central scar is seen at the place of the graft.

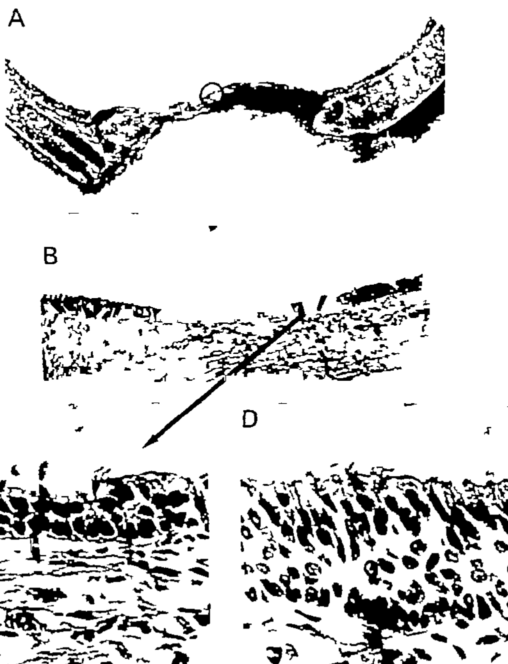


Fig 5 (*A*) Cross-section of the anterior tracheal wall. A membrane with small remnant of the grafted cartilage connects the ends of the cut tracheal rings. 6.5 (*B*) Central part f the grafted region. The ciliated columnar epithelium in the periphery decreases in height to the central part. The cilia also decreases in number from the periphery to the centrum 170. (*C*) Cuboidal and low columnar epithelium in the centrum. Only few f the cells have cilia 880 (*D*) Columnar ciliated epithelium peripherally in the grafted region 880

by any respiratory symptom during the observation time of 11 to 12 months but one dog had a tracheal stenosis.

The cartilage in the graft was almost completely resorbed when the dogs were killed. *It is possible, however that the cartilage in* the graft worked as a support during the early postoperative period and thus to some extent prevented more pronounced stenosis. Flemming & Hommerich (1968) showed that cartilage was present after 3 months when a composite graft from the auricle was used in rabbits

The dog with tracheal stenosis was given succinylcholine during the operation. It had salivation and bronchial secretion after the operation. This complication, which might be due to the fact that dogs have great sensibility to succinylcholine, probably hindered the healing. On autopsy this dog showed an almost complete rejection of the graft, and the cut ends of the tracheal rings had fallen together

The mucosa in the reconstructed tracheal wall had an epithelium which mostly was columnar with cilia, but also cuboidal epithelium was found centrally The anterior part of the nasal septum in dog has a squamous epithelium and ciliated columnar epithelium commences at the point where the maxillo-turbinate *branches (Negus, 1958) The presence of* lumnar epithelium in most parts of the wall supports the opinion, previously emphasized by Flemming & Hommerich (1968) that squamous epithelium can be transformed to columnar epithelium after transplantation to the trachea. It seems likely that an initial partial or complete necrosis of the epithelium in the graft was followed by the appearance of columnar epithelium either directly or by ingrowth from the surrounding tracheal epithelium.

No mucus transportation could be shown in the central scar of the graft except in one dog. The number of cilia was probably too small and their function might also have been poor especially when the mucosa was exposed to the lamps during filming. However the mucus transport around the scar was sufficient to prevent an accumulation of mucus below the graft. The only dog which had mucus flow over the reconstructed region had an oblique transport. This observation favours the opinion that the cilia had retained their original direction of beat, since the mucus flow goes in a dorsocaudal direction in the anterior part of the middle third of the septum, at least in man (Hilding, 1932 van Ree & van Dishoeck, 1962, Ewert, 1965) Correll & Beattie (1956) found a maintenance of the orignal ciliary direction of beat after reversal of a tracheal segment in dogs.

Whether the described method is suitable in man cannot be determined by experiments in animals only Efforts to find an ideal method for tracheal replacement have not yet been successful (Fredricksson et al., 1969) neither does the described method solve this problem. If the method is to be tried in man the technical problems of obtaining a graft of sufficient size will probably be less in man than in dogs. The nasal septum in man is more easily accessible than that in the dog, and a transection of the base of the ala nasi can be used if necessary A large piece of septal cartilage with the mucosa attached on one side can be taken preserving the mucosa on the other side The raw surface in the nasal septum can be covered by a split skin graft, similar to that used in septal dermoplasty according to Saunders (1960) for treatment of Osler's disease of the nasal mucosa. Small incisions in the cartilage may favour the apposition to the tracheal window and give a curved appearance similar to that of the tracheal wall. The resorption of the cartilage is a disadvantage If the observation by Flemming & Hommerich (1968) that the cartilage in a composite graft from the auricle remains after 3 months also is valid for a composite graft from the nasal septum, it may provide sufficient time to prevent stenosis

ZUSAMMENFASSUNG

Ein composite-graft aus Knorpel und Schleimhaut vom Nasenseptum wurde bei fünf Hunden zur Schlei-

sung eines Fensterdefektes in der zervikalen Trachea transplantiert. Atembeschwerden konnten in einer Zeitspanne von 11 12 Monaten nicht beobachtet werden. Vier Hunde wiesen am Ort des Transplantates nur eine geringe Verminderung des Trachealumens auf während bei einem Hunde eine ausgesprochene Verengerung auftrat, die wahrscheinlich ihre Ursache in einer Narkosekomplikation hatte. Nur Reste des transplantierten Knorpels waren zurückgeblieben. Die Schleimhaut hatte meistens Zylinderepithel mit Zilien, jedoch hatte der zentrale Teil oft kubisches Epithel ohne Zilien. Es ist wahrscheinlich, dass eine Umwandlung zu Zylinderepithel stattgefunden hat, weil bei Hunden gewöhnlich der vordere Teil des Nasenseptums von *Plattenepithel* bekleidet ist. Nur bei einem Hunde konnte Schleimtransport uber den zentralen Teil des Transplantatabschnittes beobachtet werden. Die Möglichkeit die beschriebene Methode auch beim Menschen anzuwenden wird diskutiert.

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MUCO-EPIDERMOID CARCINOMA OF THE PALATE

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Abstract A histological reclassification of 383 tumours in the palate showed a muco-epidermoid carcinoma in 27 cases. The relati e incidence of this type of tumour is considerably higher in the minor salivary glands of the palate than i the major salivary glands. The characteristic combination of mucous-secreting cell proliferations and epidermoid differentiation is of diagnostic significance. The tissue structure of origin is present in the terminal portion of the excretory ducts f the salivary glands and it can be concluded that the muco-epidermoid carcinomas develop from the coveri g epithelium in these ducts. The relatively greater number of such duct in the palate compared with the major salivary glands might explain the relati ely high frequency of muco-epidermoid carcinoma of the palate A histological grading of the muco-epidermoid carcinomas was based solely upon the cellular structure and the 27 muco-epidermoid carcinomas were divided into two histologically characteristic types of differentiation one hly nd one poorly differentiated. A long-term ical follow-up study showed that no patient with highly differentiated muco-epidermoid carcinoma had metastases or died of the tumour disease The question whether the highly differentiated type of muco-epidermoid carcinoma is to be regarded as benign is discussed. The prognosis of the poorly differentiated muco-epidermoid carcinomas on the other hand was shown to be extremely poor A the prognosis of highly differentiated muco-epidermoid carcinoma is so different from that of the poorly differentiated type and from that of all other malignant tumours, a histological distinction of the highly differentiated muco epidermoid carcinoma is ery important.

The characteristic histological structures of muco-epidermoid carcinoma (M E.C) were described by among others Schilling (1921) The tumour was first denoted as a well-defined entity among the salivary gland tumours by Stewart et al. (1945) and later by Linell (1948) The term refers to the biphasic structure of the tumours being composed of mucous and epidermoid cells

Tumours in the major and the minor salivary glands exhibit essentially an identical pathology *The latter tumours, however offer different* problems from diagnostic therapeutic and prognostic points of view when compared with tumours arising in the major salivary glands. *As more than 90% of all salivary gland tu*mours are localized in the major glands (e g Seifert, 1966) case series with sufficiant numbers of tumours from the minor salivary glands to make clinico-pathological correlations and prognostic evaluations possible are rare

About 60 % of intra-oral salivary gland tumours occur in the palate (Lucas, 1964 Stuteville & Corley 1967) This implies that the glands of the palate are more frequently the site of tumours then any of the other intraoral salivary glands.

In this presentation an account is given of a series of M E.C occurring in the palate Special attention has been made to the histological appearance of the tumours in relation to the clinical course of the disease

Present Series

The present study is based on an analysis of 383 histologically verified tumours of the palate in patients registered at Radiumhemmet from 1909–66

The greater part of the material was primarily classified according to a nomenclature

in which several types of tumours, not defined until recent years, were assigned to diffuse collective groups. To enable a histological–clinical correlation study to be carried out, a histological re-examination and reclassification of the tumours was made of the whole material. This was possible since slides and/or tissue blocks are filed at the Department of Tumour Pathology

The histological re-examination of the palatal tumours disclosed a salivary-gland tumour in 170 patients benign in 95 and malignant in 75 Tumour structures characteristic of M.E.C. were identified in 27 patients, who had been treated during the 1931–63 period.

All 27 patients with a M.E.C. of the palate were followed up for at least 5 years. The observation period was reckoned from the date of the first histological verification of the tumour

The prognosis of M.E.C. of the palate was investigated by studying the rate of local recurrence metastasis, mortality and the survival rate

The survival rate is based on determinate groups Indeterminate patients, i.e those lost to follow-up and those who died without signs of tumour disease, are excluded.

Thus the grade of malignancy of M.E.C. was evaluated by studying the determinate survival rate, which is based on the mortality in the tumour disease. A long observation period is necessary to obtain reliable prognostic figures. In the present series the follow-up period ranged from 5 to 37 years.

Histological Features

The muco-epidermoid carcinomas in the present series exhibited the histological structure frequently described for tumours of this type occurring in the major salivary glands (e g. Stewart et al., 1945 Linell 1948 Foote & Frazell, 1954 Eneroth, 1964 Eneroth et al 1967 Jakobsson et al 1968) Thus the tumours were composed of the three different types of cells, epidermoid cells, mucous-secreting so-called goblet cells, and a less identifiable cell type commonly denoted as intermediate cells. All cell types were mixed in varying proportions in the different tumours and also in different areas within the same tumour The diagnosis mucoepidermoid carcinoma as such involved no major difficulties. All three cell types were demonstrable in all cases.

The tumours were graded as highly differentiated or poorly differentiated according to Stewart et al. (1945) Linell (1948), Foote & Frazell (1954) and Eneroth (1964). The grading was based upon the degree of maturation of the cell components of the tumours. Cytological structures such as cellular polymorphism variation in nuclear size and chromatin content, frequency of mitotic figures and degree of cellularity were taken into account for the estimation of the degree of the differentiation of the tumours. The mode of growth in the form of demonstrable lack of encapsulation or presence of invasive growth (as suggested by Jakobsson et al., 1968) was not considered for the histological grading of the tumours.

In the present series of 27 cases of M.E.C of the palate 21 were found to be of the highly differentiated type and 6 showed structures corresponding to poorly differentiated tumours.

In the highly differentiated tumours there was no predominance of any one of the three cell types, epidermoid, mucous-secreting or intermediate. All three types showed little or moderate cellular polymorphism and mitotic figures were rare A varying structure in different areas was typical for the tumours (Fig. 1) In numerous cases solid cords with predominance of intermediate and epidermoid cells were found (Fig. 2) In all tumours these structures were mixed with such where mucous-secreting cells dominated (Fig. 3) Adenomatous differentiation was frequently found, mostly however combined with different degree of epidermoid differentiation (Fig. 4) Cystic structures of varying dimensions were found in all 21 cases. In no fewer than 10 cases the tumours were predominantly cystic, partly lined with a

Fig. 1. Muco-epidermoid carcinoma of the palate. Highly differentiated type with varying structures in different areas. Photomicrograph. × 70.



Fig. 2. The same case as Fig. 1. A ca with predominance of intermediate and epidermoid cells. Photomicrograph. × 150.

Fig. 3 The same case as Fig. 1 Areas with mucous-secreting (goblet) cells. Photomicrograph. × *150*

Fig. 4 Muco-epidermoid carcinoma f the palate. Highly differentiated type with epidermoid structures. Photomicrograph. × 150.

Fig. 5. Muco-epidermoid carcinoma of the palate. Highly differentiated tumour of the cystic type. Photomicrograph. × 150.

Fig. 6. Muco-epidermoid carcinoma of the palate. Poorly differentiated type. Predominantly epidermoid structures with considerable cellular polymorphism. Photomicrograph. × 150.

single layer of mucinous cells (Fig. 5) The stroma of the tumours showed only moderate fibrosis and no myxoid or chondroid differentiation could be found which would suggest a pleomorphic adenoma.

The highly differentiated tumours developing in the palate had no distinct fibrous capsule In fact the absence of a tumour capsule made it impossible to evaluate whether invasive growth was present or not. From a purely morphological standpoint all tumours exhibited growth in the adjacent tissues simulating invasive growth.

In the poorly differentiated tumours the epidermoid component dominated the tumour structure The squamous proliferations exhibited marked cellular polymorphism and numerous mitotic figures (Fig. 6) Only little or moderate keratinization could be demonstrated. Areas with intermediate cells were always found but mucinous cell components were only present in minute areas. In 2 cases this structure had to be sought by serial sectioning of the blocks. The presence of mucinous cells is, however essential for the differential diagnosis toward epidermoid carcinoma and such cells were demonstrable in all 6 cases. The tumour cords were predominantly solid and showed only little tendency to adenomatous formations. Larger cysts were missing. All tumours were invasive, in 2 cases with growth into the underlying bone of the hard palate.

Lymph node metastasis were found in 2 cases. In both, the metastasizing tumour structure was entirely epidermoid in character

Clinical Features

The highly differentiated type of M.E.C. occurred in 7 men and 14 women, the poorly differentiated type in 4 men and 2 women. The mean age of the patients with a highly differentiated tumour was 44.3 years, 17 of the 21 patients being younger than 55 years. The corresponding figure in patients with a poorly differentiated tumour was 68 8 years, all 6 patients being older than 55 years.

The mean duration between appearance of the first symptoms of the tumour and its histological verification was 2.3 years in the group of highly differentiated M.E.C. and 0.5 year in the group of poorly differentiated M.E.C. (the duration being less than 1 year in all 6 cases in this group)

A lump in the palate was the commonest symptom of the tumour disease It was detected by the patient himself in 16 cases, by a dentist in 3 patients who complained of difficulty in wearing their dentures, and accidentally by a dentist in another 4 patients without complaints

Pains occurred in 5 patients, 4 of whom had a poorly differentiated tumour Nasal discharge and blocked nasal passage was present in 2 cases. In 22 cases the tumour was localized to the hard palate in 3 to the soft palate and in 2 cases to both the hard and soft palate. Extension beyond the palate was present in 6 cases (2 cases with a highly differentiated tumour and 4 cases with a poorly differentiated tumour) 5 of them with primary localization to the hard palate and one to the soft palate. Extension from the hard palate occurred to the nasal cavity and maxillary sinus and extension from the soft palate to the base of the tongue

In 20 cases the diameter of the tumour was larger than one centimetre and in 10 of these cases larger than 2 cm. The surface of the tumour was stated to be ulcerated in 3 of the 21 cases of highly differentiated tumour and in 4 of the 6 cases of poorly differentiated tumour

X-ray examination of the palate in 20 cases showed bone destruction in 2 cases with poorly differentiated M.E.C and suspicious bone destruction in 1 case with highly differentiated M.E.C

At the first examination, palpatorily definite cervical lymph node metastases were considered to be present in only 1 case The primary tumour in this case was a poorly differentiated M.E.C

Treatment

The treatment has been surgery irradiation, or a combination of these two forms of therapy The principles of the treatment, however have

varied during the long period the tumour material comprises.

The surgical treatment consisted of extirpation of the tumour in 23 cases, combined with resection of the bony palate in 2 cases and with electrocoagulation in 6 cases In the remaining 4 cases the surgical procedure was small—probably only a biopsy

Radiotherapy was given in 18 cases, preoperatively postoperatively or both pre- or postoperatively in 5 11 and 2 cases respectively No irradiation was given in 9 cases all with a highly differentiated M.E.C.

Prognosis

Recurrence

No local recurrence was found in the 21 patients with a highly differentiated M.E.C., whereas 2 recurrences were found both within 1 year in the 6 patients with a poorly differentiated M.E.C. In another 3 cases with poorly differentiated M.E.C. the tumour exhibited a progressive growth despite treatment.

Metastasis

Regional lymph node metastases were demonstrated in 2 cases, both with a poorly differentiated M E.C In 1 case the metastases were observed already at the first examination whereas in the other case they appeared after years Distant metastases were not observed in any case

Survival

The prognosis was determined chiefly by studying the mortality in the tumour disease and the determinate survival rate

As previously stated the 27 muco-epidermoid carcinomas of the palate were re-examined and divided into two histologically characteristic types of differentiation, a highly differentiated and a poorly differentiated type Of the 21 patients with a highly differentiated tumour none died of the tumour disease and 5 of intercurrent disease, whereas the corresponding figures in the 6 patients with a poorly differentiated tumour were 5 patients who died of

Table I *5–35 year follow-up study of 27 patients with muco-epidermoid carcinoma of the palate*

Histological features	No. of patients	Died of Tumour disease	Died of Intercurrent disease
Highly differentiated	1	0	5
Poorly differentiated	6	5	1
Total	27	5	6

the tumour disease and 1 of intercurrent disease (Table I)

In the group of highly differentiated M.E.C 21 patients had been under observation for at least 5 years, 18 for at least 10 years, 13 for at least 15 years and 7 for at least 20 years (Table II) No patient was lost to follow up and altogether 3 patients died of intercurrent disease during the 20-year follow-up period. As no patient in this group died of the tumour disease the 5- 10- 15- and 20-year determinate survival rate of highly differentiated muco-epidermoid carcinoma is 100%

In the group of 6 patients with poorly differentiated M E.C., 5 patients died of the tumour disease within 3 years and 1 patient of intercurrent disease 8 years after treatment. The 5 year and 10-year determinate survival rate of poorly differentiated muco-epidermoid carcinoma is 16 7 and 0% respectively The 5-year determinate survival rate of M E.C without taking into account the histological subgroups is 81

Discussion

Earlier investigations of M E C in the minor salivary glands have as a rule been based on small tumour series with short observation time The present material is proportionately large and the follow up period long and therefore the histological and clinical study of this material is of value for assessing the biological properties of this type of tumour in the minor salivary glands.

Table II. *5–20 year follow-up study of 21 patients with highly differentiated muco-epidermoid carcinoma of the palate*

Observation period (years)	No. of patients	Died of: Tumour disease	Died of: Intercurrent disease	Lost to follow-up	Determinate survival rate: No. of patients	No. of survivals	Survival ( )
5	21	0	1	0	20	20	100
10	18	0	2	0	16	16	100
15	13	0	3	0	10	10	100
20	7	0	2	0	5	5	100

Muco-epidermoid carcinoma constitutes an incidence of 10% of all intra-oral minor salivary gland tumours and the palate is the most frequent site (Chaudhry et al. 1961) Next to the parotid gland the palate is the commonest localization of M.E.C (Bhaskar & Bernier 1962)

The relative incidence of M.E.C. is, however higher in the glands of the palate than in the parotid and submandibular glands Thus the 27 cases of M.E.C. of the palate in the present series comprise 15 8% of all salivary gland tumours in this location. In previous investigations of tumours of the major salivary glands, M.E.C. was found to account for 3 7% of all salivary gland tumours of the parotid gland (Jakobsson et al., 1968) and for 3 6% of those of the submandibular gland (Eneroth & Hjertman, 1967 Eneroth et al., 1967) Of the malignant salivary gland tumours of the palate, parotid gland and submandibular gland, M.E.C. comprised 36 0 21 1 and 9 7% respectively according to the same authors.

The characteristic histological appearance of the muco-epidermoid carcinomas suggests a well-defined histogenesis Mucous-secreting cell proliferations are found in other types of salivary-gland tumours such as papillary adenocarcinomas. Epidermoid differentiation is a common feature in pleomorphic adenomas The rather characteristic combination of the two components found in muco-epidermoid carcinomas is, however of diagnostic significance. The tissue structure of origin is present in the terminal portion of the ducts of the salivary glands. The covering epithelium of these ducts (Fig. 7) contains both cellular components, one superficial mucous layer with typical goblet cells and one underlying layer with smaller cells, similar to the intermediate cells found in the muco-epidermoid carcinomas These cells often show a tendency to epidermoid differentiation It can thus be assumed that the muco-epidermoid carcinomas develop from the covering epithelium in the excretory ducts. The relatively high frequency of muco-epidermoid carcinomas among salivary gland tumours arising in minor salivary glands compared with those in the major glands might be explained by the relatively greater number of such ducts in areas with numerous individual small salivary glands, such as in the palate.

The histological grading of the muco-epidermoid carcinomas in the present series was based solely upon the cellular structure of the tumour tissue. No well-defined fibrous capsule could be demonstrated in the 27 cases and thus lack of encapsulation makes any evaluation of presence or absence of invasive growth entirely subjective or hypothetical. The muco-epidermoid carcinomas originating in the palate differ in this respect from those developing in the major salivary glands (Eneroth et al., 1967 Jakobsson et al., 1968) However it may be emphasized that histological grading of any tumour should be kept apart from the relation of the tumour tissue to adjacent tissues which rather should be registered as a histological staging. A highly malignant tumour may also be encapsulated and still give rise to metastases. On the

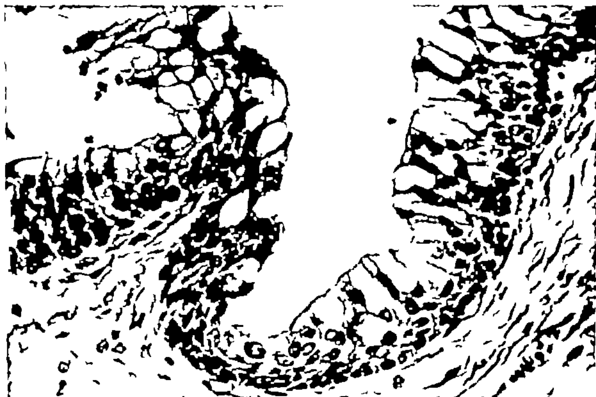


Fig 7 Duct from normal minor salivary gland of the palate. Mucous-producing (goblet) cells and underlying intermediate cell types. Photomicrograph. 400.

other hand, as in the 21 cases of highly differentuated low grade malignant M E.C in the present series, the tumour may lack a well-defined capsule without showing signs of malignant behaviour in the form of metastases. The clinical course of M E.C in the palate presented in this paper seems to give support to the reliability of the histological grading applied based solely upon the cellular structures of the tumour population itself

The mean age of patients with poorly differentiated M E.C was 24.5 years higher than of patients with highly differentiated M E.C A difference in mean age between patients with highly and poorly differentiated adenoid cystic carcinoma of the palate has been observed (Eneroth et al., 1968) but in this type of tumour the mean age was higher in patients with highly differentuated types of tumour

Patients with a poorly differentiated M.E C had a short duration of their symptoms before the tumour was histologically verified, in all 6 cases less than 1 year In this type of M E C pain was a common symptom which probably explains why the patients so soon consulted a doctor In patients with a highly differentiated M E C pain was very rare and the duration of the first symptoms of the tumour disease usually a lump in the palate was considerably longer Another difference between the appearance of the two types of M E.C. was the frequency of ulceration of the mucous membrane over the tumour which was much higher in the poorly differentiated type The important role of the dentist in discovering intra-oral tumours is shown by the fact that 7 of the 27 tumours in the present material were detected by a dentist.

About 4 5 of the muco-epidermoid carcinomas in the present series were located to the hard palate A similar difference was noticed between the hard and soft palate with respect

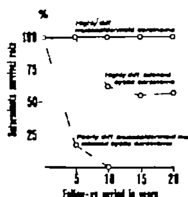


Fig 8 Relation between follow-up period and determinate survival rate of highly and poorly differentiated muco-epidermoid and adenoid cystic carcinoma of the palate.

to adenoid cystic carcinoma (Eneroth et al., 1968) Of the epidermoid carcinomas of the palate on the contrary only 2/5 were situated in the hard palate (Hjertman & Eneroth, 1970) This difference as regards the localization to the hard and soft palate of salivary-gland tumours and epidermoid carcinomas is in accordance with the observations of Martin (1942)

The correlation study between histological structure and clinical course of M.E.C. of the palate showed a clear difference in prognosis between the two histologically different subgroups. In the group of highly differentiated muco-epidermoid carcinomas no local recurrences and no metastases were observed and no patient died of the tumour disease during the very long observation period. In the group of poorly differentiated muco-epidermoid carcinomas, however both local recurrences and metastases occurred and 5 of 6 patients died of the tumour disease all within 3 years. Consequently the obvious difference in prognosis between the highly and poorly differentiated M.E.C of the palate justifies a classification into a low grade malignant and a high grade malignant type on the basis of the differentiation of the tumours The high grade malignant type is associated with an exceedingly poor prognosis, whereas that of the low grade malignant type is favourable

Earlier studies of tumours of the submandibular gland (Eneroth et al., 1967) gave results in support of the justification of classifying muco-epidermoid carcinomas into two subgroups on the basis of the histological differentiation, a poorly differentiated type with poor prognosis (no patient alive after 5 years) and a highly differentiated type with favourable prognosis (no patient died of the tumour disease)

On the basis of these studies of M.E C. of the palate and those of the submandibular gland one might consider highly differentiated M.E.C. as benign In a study of tumours of the parotid gland, however 2 patients with a highly differentiated M.E.C died of the tumour disease (Jakobsson et al., 1968) There is, however still no general agreement about the controversial question as to whether highly differentiated muco-epidermoid carcinomas should be considered as true malignant neoplasms

Muco-epidermoid carcinoma and adenoid cystic carcinoma are the dominant malignant tumours in the minor salivary glands of the palate constituting 36% and 49% respectively (Eneroth et al., 1968) As was shown in our study of adenoid cystic carcinoma of the palate there is an obvious difference in prognosis between the highly and poorly differentiated type of this tumour Similarly as has been shown in the present study there is a clear prognostic difference between highly and poorly differentiated M.E.C of the palate. A comparison of the prognosis of adenoid cystic carcinoma and muco-epidermoid carcinoma of the palate is made in Fig. 8 The poorly differentiated types of the two tumours do not differ in prognostic respect, whereas the highly differentiated types differ prognostically The prognosis of the highly differentiated muco-epidermoid carcinomas can be satisfactorily estimated after a follow-up period of 5 years. No patient died of the tumour disease within this time or during the continued follow up period. The prognosis of the highly differentiated adenoid cystic carcinomas cannot, however be definitively established after a follow up period of only 5 years The 5-year determinate survival r is

type of tumour is 100 %, the 10-year determinate survival rate, however, only 62 %.

The above-described differences in prognosis illustrate the importance of a histological distinction of the highly differentiated muco-epidermoid carcinomas of the palate from poorly differentiated muco-epidermoid carcinomas on the one hand and from other malignant tumours on the other.

ZUSAMMENFASSUNG

Eine histologische Umklassufizierung von 383 Tumoren im Gaumen ergab ein Mucoepidermoidkarzinom in 77 Fällen. Das relative Vorkommen dieses Tumortyps ist in den kleinen Speicheldrüsen im Gaumen bedeutend höher als in den grossen. Die charakteristische Kombination von schleimproduzierenden Zellproliferationen und epidermoider Differenzierung ist von diagnostischer Bedeutung. Das Ursprungsgewebe ist in dem terminalen Teil der Speicheldrüsengänge vorhanden. Daraus kann man schliessen, dass sich die Mucoepidermoidkarzinome aus dem Epithel dieser Gänge entwickeln. Die relativ grossere Anzahl von solchen Gängen im Gaumen, verglichen mit den grossen Speicheldrusen, kann die relativ höhere Frequenz von Mucoepidermoidkarzinomen im Gaumen erklären. Eine histologische Gradierung der Mucoepidermoidkarzinome gründet sich nur auf die zellulare Struktur. Die 77 Mucoepidermoidkarzinome wurden in zwei histologisch charakteristische Typen der Differentzierung eingeteilt, in einen hoch- und einen niedrigdifferenzierten Typ. Eine langfristige klinische Nachkontrolle ergab, dass kein Patient mit einem hochdifferenzierten Mucoepidermoidkarzinom Metastasen hatte oder an der Tumorkrankheit starb. Die Frage, ob der hochdifferenzierte Typ des Mucoepidermoidkarzinoms als benign zu betrachten ist, wurde diskutiert. Die Prognose der niedrigdifferenzierten Mucoepidermoidkarzinome dagegen war äusserst schlecht. Da die Prognose des hochdifferenzierten Mucoepidermoidkarzinoms so ganz unterschiedlich zu der des niedrigdifferenzierten Typs und zu allen anderen malignen Tumoren ist, ist eine histologische Distinction der hochdifferenzierten Mucoepidermoidkarzinome sehr bedeutungsvoll.

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ATTEMPTS AT EVALUATION OF THE FUNCTION OF VARIOUS LARYNGEAL MUSCLES IN THE LIGHT OF MUSCLE AND NERVE STIMULATION EXPERIMENTS IN MAN

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Abstract. Muscle and nerve stimulation experiments have been carried out on a series of 12 human larynges with malignant disease of essentially unilateral involvement. In the course of total laryngectomy tests were pplied to several internal and external laryngeal muscles as well as the ecurrent and superior laryngeal nerves on both sides. Muscle stimulation experiments were carried out also on the recently excised larynx. The effects were studied on the relatively unaffected side, where vocal fold mobility was pparently free. Stimulation of the sterno-thyroid muscle constantly led to dilatation of the laryngeal inlet and widening of the glottis. Abduction of the vocal fold occurred only in 50% of the cases (3 out of 6) on stimulation of the posterior crico-arytenoid muscle. Various explanations of these phenomena are discussed. Stimulation of the recurrent laryngeal nerve produced adduction of the vocal fold to the paramedian plane. In two experiments out of seven, however stimulation of the recurrent laryngeal nerve led to abduction of the vocal fold. In no instance could repeated stimulation of this nerve produce two different responses, abduction and adduction, in the same experiment. Evidence is presented of the existence of both sensory and motor bundles in the recurrent laryngeal nerve. Adduction of the vocal fold to an intermediate position was noticed on stimulation of the superior laryngeal nerve. This was shown to be mediated through its external branch, the effector muscle being the crico-thyroid.

The most ancient premordium of a larynx is represented by the sphincter-like structure found at the entrance of the primitive respiratory tract in the lung-fish (Negus, 1949) The main function of the sphincter is to protect this simple lower respiratory tract. The process of evolution of laryngeal structure in the animal kingdom led to the introduction of cartilages, and accordingly the single sphincter was divided into separate muscles. This structural maturation enabled the larynx to cope with the new functions acquired in terrestrial life

No significant disagreement remains about the broad lines of laryngeal function. Opinions differ however concerning the details of laryngeal activities and the way they are achieved. Phonation, particularly pitch control, has been studied more elaborately than the vital opening and closing mechanism of the larynx. The understanding of these two movements is the key to explain a great deal of laryngeal function and dysfunction. Some recent publications, however have focused attention on several doubtful aspects of the mechanics of these two movements (Frable, 1961 von Leden & Moore 1961 Ardran & Kemp, 1966)

Several studies have been carried out on recurrent nerve stimulation, in experimental animals as well as in man. There has been some inconsistency of the res [illegible] On stimulation of the canine recu[illegible] geal [illegible] Brewer & Dana (1963) [illegible] ura (1[illegible] Hast (1966) [illegible] aken [illegible] (1968) observed that with a c[illegible] ulus

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Fig 1 After opening the pharynx, the epiglottis is shown tilted downwards and forwards by vulcellum. The four ribbons mark the dissected superior and recurrent laryngeal nerves on both sides.

tude, low frequency (20/sec) induced abduction movement of the vocal folds. With an increasing stimulus frequency (30–70/sec) there was an increasing adduction of the vocal folds, and complete closure of glottis was reached at the highest frequency level. Murtagh (1945) and Williams (1951) on the other hand, working with goats and humans respectively ob- only adduction movements of the vocal folds on minimal stimulation of the recurrent laryngeal nerves. The effect of changing the impulse amplitude has been studied by Seymour & Henry (1954) on cats, and by Capps (1958) on humans In their experiments there was a tendency to adduction at low voltage, while steadier abduction resulted in response to an increase of the strength of the stimulus Some of the evident controversies can, at least in part, be explained on the variability of the experimental models Seymour & Henry (1954) also felt that in this particular problem animal results cannot be applied with any certainty to man"

In an attempt to understand the mechanism of action of laryngeal muscles series of stimulation experiments have been carried out, in which the recurrent laryngeal nerves and the superior laryngeal nerves were stimulated. Several internal and external laryngeal muscles were also subjected to the test. Our main concern has been to observe the effect of these stimulations on vocal fold position and configurations. These experiments were decided to be carried out in man, since no experimental animal could fulfil the requirements of the investigation. One of the foundations of speech, which is a human characteristic, lies in the larynx, which has reached a high degree of developmental maturity If the details of function of the laryngeal muscles in man are to be studied, little place is actually left for animal experiments. The differences in structure and expected function are too fundamental to allow any valid or significant comparative study

A. *Muscle Stimulation Experiments*

1 *The larynx in situ*

The present series of experiments has been attempted on patients subjected to neck operations, mostly total laryngectomy for treatment of malignant tumours. In each case only one or two experiments could be carried out, thus minimizing the time spent on these specific procedures. A primary interest was devoted to the investigation of the strap muscles of the neck, especially the sterno-thyroid. The individual members of the strap muscles were dissected out and identified. In order to reproduce a condition as similar as possible to that which exists in the intact neck, the muscles attachments were left untouched, so that their effect on the thyroid cartilage and hyoid bone should not be significantly interfered with. For this reason the only channel left for observation of the vocal folds was through direct laryngoscopy utilizing a Magill laryngoscope. In these cases the anaesthetics were delivered via a tracheostomy cannula, thus leaving the larynx free for observation. Endotracheal anaes

thesia was used routinely utilizing halothane nitrous oxide and oxygen, with thiopentone and succinylcholine for induction.

Stimulation of the small internal laryngeal muscles could be achieved only after laying open the pharynx and tilting the larynx forward thus allowing direct inspection of the interior of the larynx (Fig. 1) Under these conditions the muscles could be reached fairly easily and at the same time effects on the vocal folds position could be observed directly

The muscles were stimulated by a square wave impulse (0.5 msec duration) generated by a DISA Myostim or a DISA Ministim type 14E01 The square wave was delivered to the muscle by a DISA Teflon-coated needle electrode. Sterility precautions were observed strictly at all stages of the experiment. The impulse amplitude was adjusted to be near threshold. Higher amplitudes could give rise to significant contractions of distant muscles due to volume conduction. The impulses were applied at a frequency starting from 1/sec and gradually rising to 100/sec.



Fig. 2 The recently severed larynx showing the active electrode inserted into the left posterior crico-arytenoid muscle The indifferent electrode is placed in the site of the pre-epiglottic body

2. *The excised larynx*

A freshly excised larynx can be utilized in muscle stimulation experiments for a fairly large number of tests (Fig. 2) The limitations of the experiment lie in the fact that metabolites accumulate and render the response of the muscles very poor and unfit for comparative study All required tests could be tried within a short time and with a limited number of stimuli that did not even necessitate perfusion of the larynx in saline The same type of stimulus and apparatus was used to test the posterior crico-arytenoid, thyro-arytenoid and crico-thyroid muscles.

It should be emphasized that the interpretations of the results in both types of experiment were based only on observations made on the freely mobile vocal fold. Cases with bilateral involvement and marked limitation of movement of both vocal folds were considered unfit for the purpose and were discarded.

Results

The present results were reached upon analysis of observations made on 12 patients subjected to total laryngectomy The lesion was mainly a unilateral malignant affection of the larynx. The cases were operated upon either for recurrence of the tumour after primary radiotherapy or because of primary failure of this line of treatment. Mobility of the apparently unaffected vocal fold was reasonably free.

The results shown in Table 1 highlight one salient feature—the inconsistency of the results, which may be due to variations in the extent of the pathological process, in spite of the apparent stability of the experimental conditions. The variability of the results amounts to complete contradiction regarding the posterior crico-arytenoid muscle. It was noted, however that for the individual case there was a tendency to uniformity of action of all internal laryngeal muscles whether so-called

Table I

Stimulus	No. of expts.	Muscle	Results: Add.	Results: Abd.
5–15 V	6	Post. cricoaryt.	3	3
0.5 msec	7	Cricothy	6	1
Freq	3	Thyroaryt.	3	
1 100/sec	2	Interaryt.	2	
	5	Sternothy	Widening of laryngeal inlet and abduction of vocal folds in all experiments.	

abductors or adductors, either to abduct or adduct the vocal folds at the crico-arytenoid joint. The most consistent result was the dilatatory effect consequent upon stimulation of the sterno-thyroid muscle. This effect was found to be specific for stimulating this muscle By using an impulse at threshold intensity the effect of dilatation and descent of the larynx was noticed only when the stimulatory electrode was applied to the muscle itself. No effect was noticed when stimulating at the mid line between the muscles on each side Crico-thyroid muscle stimulation produced, in addition to adduction, a distinct crico-thyroid approximation.

B *Nerve Stimulation Experiments*

In the course of the operation (total laryngectomy) the recurrent laryngeal and the superior laryngeal nerves on both sides were dissected out and identified (Fig. 3) No stimulation was attempted before the pharynx was opened and the interior of the larynx was visualized. At this stage the effects of stimulation of the various nerves on the vocal folds could be easily observed. The same apparatus and stimulating impulses as in muscle stimulation experiments were employed Special emphasis was made on studying the influence of changing the frequency of stimuli. The frequencies used were in the range of 1/sec, gradually increasing up to 100/sec Trains of stimuli were used as well, of 10–100 impulses/sec, of varying duration of 50–1 000 msec Threshold stimulation was preferred to supramaximal impulses in order to scrutinize the individual specific effect of each nerve The interpretation of vocal fold responses was made, like in the muscle stimulation experiments, by at least two observers.

Table II

Stimulus	No. of expts.	Nerve	Results
5–15V 0.5 msec Freq 1 100/sec	7	Recurrent Laryngeal	4 Add to paramedian position. 2 Abd. 1 Abd. at low freq (1 10/sec) Add. at high freq (10–100/sec) (2 Add. on both sides)
	6	Superior Laryngeal External branch intact	4 Add. to intermediate position
		External branch severed	1 Weak add. 1 No response

Fig 3 The left recurrent laryngeal nerve is dissected out close to the point of entrance into the larynx near the crico-thyroid articulation (*arrow*). Anaesthetics are administered via the tracheal cannula.

Results

The observed effect of stimulation of the laryngeal nerves on vocal fold position was more or less constant. As shown in Table II stimulation of the recurrent laryngeal nerve led to powerful adduction of the vocal fold to a paramedian position, with an estimated chink of 3–4 mm. In 2 cases out of 7 dilatation of the glottis was noticed consequent upon recurrent nerve stimulation. In only 1 case did the effect of stimulation vary with changing of the frequency of impulses. At a low frequency rate (1–10/sec) there was dilatation, while at higher frequencies (10–100/sec) the result was a narrowing of the glottis It has been an almost constant finding that the vocal fold was adducted to the paramedian position on stimulation of the recurrent nerve, both on the ipsilateral and contralateral side

Stimulation of the superior laryngeal nerve constantly led to adduction of the vocal fold. This adducting effect, however was less complete than that produced by recurrent nerve stimulation. The vocal fold on the mobile side was brought to an intermediate position, with an estimated chink of 5–6 mm.

DISCUSSION

The details of the mechanism of action of the internal laryngeal muscles are based on mechanical analysis of the muscles pull on the crico-arytenoid and crico-thyroid joints. Seeing the nonreliability of the conventional crico-arytenoid model one would expect a similar nonreliability of the traditional concept of the action of the arytenoid muscles (Kotby & Haugen, 1970 *a*, *b*)

It is suggested that, in the *light of the recently introduced concept of crico-arytenoid movement*, the posterior crico-arytenoid muscle is not in a favourable mechanical situation to effect significant abduction at this joint. Even under the conventional consideration of the crico-arytenoid joint, an efficient lateral displacement of the arytenoid mass by the posterior crico-arytenoid muscle has been discounted (Fink et al., 1956) Moreover individual anatomical variations of the crico-arytenoid disposition may lead to significant variation in vocal fold movement (Zenker 1964) These variations can explain, at least partially the inconsistency of our results concerning posterior crico-arytenoid muscle stimulation.

In our experiments, stimulation of the posterior crico-arytenoid muscle did not lead constantly to abduction. There was an equal tendency to abduction and adduction. The adducting effect on stimulation of the posterior crico-*arytenoid muscle was a specific one.* When the active electrode was applied, at threshold intensity at an indifferent site away from the muscle, no effect on the vocal fold was noticed, while adduction was still detectable at the same intensity when the electrode was placed on the muscle In this connection, every part of the muscle, whether of transverse or oblique fibre, produced adduction of the vocal fold. As a confirmatory experiment, all muscular attachments to the arytenoid cartilage were severed, apart from the posterior crico-arytenoid muscle, whose stimulation still gave rise to adduction. This observation may be taken as a challenge to the consideration of this muscle as the sole abductor

The concept we hold at present for the mode of action of the small internal laryngeal muscles is that these are primarily postural muscles, acting individually in a common system of forces. The resultant of these forces decides the position length and configuration of the vocal folds An important modifying factor for this outcome is the configuration of the crico-arytenoid joint, whose displacement provides *the main effector of vocal fold movement* Accordingly it may be possible that stimulation of a certain muscle may lead to either an apparent abduction or adduction of the vocal fold, depending on the detailed anatomic variations of the joints Stimulation of a single muscle is in fact quite different from what actually occurs during life Laryngeal muscles rarely act singly A complex and delicate balance of the

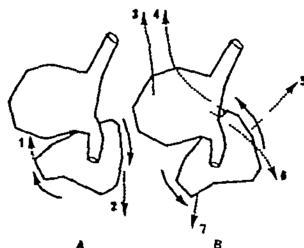


Fig 4 1 crico-thyroid muscle 2 oesophagus, 3 thyro-hyoid muscle and connective tissue 4 *functional chain*, 5 crico-pharyngeous muscle 6 sterno-thyroid muscle 7 tracheal tension. (*A*) All forces operating in front of the axis of the crico-thyroid joint, on the cricoid in an upward direction, and all forces operating behind this axis downwards, lead to closure of the visor and elongation of the vocal folds. (*B*) All forces active in front of this axis, which pull the cricoid cartilage downwards, and those effective behind the axis which pull in an upward direction, will open the visor and shorten the vocal folds. (Modified from Zenker 1964)

laryngeal structures is constantly maintained by the simultaneous activity of several internal and external muscles.

From the anatomical point of view the ex l laryngeal muscles are well defined to one point of attachment on the laryngeal skeleton. From the functional view-point it is hard to keep to this restricted definition. Muscles acting on the base of the tongue are expected to have some influence on the glottis, since they move the upper end of the *functional chain* of arytenoid, ary-epiglottic fold, epiglottis, hyoid bone and tongue. This anatomical arrangement is considered by Zenker (1964) to influence the glottis through its effect on the crico-thyroid joint (Fig. 4) Movement at this joint is mainly in the form of rotation around a transverse axis, which passes the centers of both joints. This allows the lower border of the thyroid cartilage and the upper border of the cricoid arch to be approximated and separated in a visor-like mechanism. With narrowing of the visor the vocal cords are elongated and generally adducted, and the reverse occurs on widening of this visor-like system. From Fig. 4 it can be seen that contraction of the *functional chain* will ultimately lead to shortening and abduction of the vocal folds.

Stimulation of the sterno-thyroid muscle, which is a depressor of the thyroid cartilage, led in all cases to dilatation of the glottis and, even more constantly to dilatation of the laryngeal inlet. Previous electromyographic findings (Fink et al., 1956 Kotby 1967) demonstrated significant electrical activity in the sterno-thyroid muscle during inspiration. The *glottic dilatatory effect of sterno-thyroid stimulation* can be explained on the basis of the assumption of Fink et al. (1956), that the descent of the larynx causes a stretching of the pre-epiglottic body in the anterior wall of the larynx, thus increasing the antero-posterior diameter during inspiration Moreover during descent of the larynx the folds on its lateral walls become stretched and unfolded in a way similar to the walls of a pair of bellows In addition, this laryngeal depressor imparts a stretching force in a downward direction on the ary-epiglottic fold. In other words, the lower end of the *functional chain* is pulled downwards while the upper end is fixed. The ary-epiglottic stretch will produce an upward and outward pull on the arytenoid cartilages, which are drawn laterally carrying the vestibular and vocal folds away from the middle line. This effect of pull on the *functional chain* is in accordance with observations of Ardran & Kemp (1966) on the mechanics of the crico-arytenoid joint. In support of this assumption Zenker (*1964*) suggested that the arrangement of the mucous membrane and connective tissue of the larynx allows any vertically applied force to be effective as a glottis opener There are however not a few observers who have described a significant influence of the external laryngeal muscles, in man, on phonatory adjustments of the larynx (DuBois-Reymond & Katzenstein, 1909 Katzenstein, 1922 Schilling, 1940 Arnold, 1947).

The results of the present series of experiments give supportive evidence to the assumption that the external laryngeal muscles may play a significant part in moving and adjusting the vocal folds.

Our results of nerve stimulation experiments, though showing some inconsistency concerning recurrent nerve stimulation, demonstrate certain rather distinctive trends. In no instance could repeated stimulation of the recurrent laryngeal nerve produce two different responses, abduction and adduction, in the same experiment. Apart from one single instance variation in the stimulus specifications as regards frequency and intensity gave no change of the response to stimulation. In this single experiment low frequency stimulation, 1–10/sec, led to abduction, while high frequency stimulation, 10–100/sec, led to adduction of the vocal folds. This observation, though only once recorded, is in accordance with the results of Nakamura's experiments (1964) on dogs.

Such results do not support the notion that within the recurrent laryngeal nerve there exist two different and distinct bundle systems, abductor and adductor On the other hand, it does not imply a homogeneity of the recurrent laryngeal nerve but at least, if two bundle systems exist they should be thoroughly intermingled If the abductor fibres, either from position, size or inherent quality are more susceptible than the adductors to lesions affecting the recurrent laryngeal nerve, then they should also *respond first* to minimal stimulation (Williams, 1951) Stimulation of the recurrent laryngeal nerve with stimuli at threshold values showed on the contrary a marked tendency to adduction. There were, however two experiments out of seven in which the general tendency of recurrent laryngeal nerve stimulation was towards abduction. This can be explained in part as being a result of anatomical variations in the crico-arytenoid joint. There is, however no conclusive explanation for the factors that jeopardized the adductor elements in the system of forces acting on the crico-arytenoid joint in these experiments.

These observations, as well as accumulating clinical evidence (Jackson & Jackson, 1934 Williams, 1951), failed to support Sir Felix Semon's observations on the recurrent nerve affection. He assumed the existence of a distinct abductor bundle within the recurrent laryngeal nerve and claimed the proclivity of the abductor fibres of the recurrent laryngeal nerve to become affected sooner than the adductor fibres " (Semon, 1881) It may be that the apparent vulnerability of the abductor fibres, or in other words the apparent abduction failure, is due to the end result of the disturbed equilibrium of the system of forces acting on the crico-arytenoid joint, including the joint itself. The disturbed system of internal laryngeal muscles supplied by the affected recurrent nerve, together with the fine structural variations of the joint, may play an important role in deciding the position of the paretic vocal folds. In addition, the external laryngeal muscles are expected to play a modifying role in this final outcome

There is an evidence in the presented results of the presence of sensory and motor bundles in the recurrent laryngeal nerve Stimulation of one recurrent laryngeal nerve produced adduction of the homolateral and contralateral vocal fold in two experiments out of seven. Since there is evidence that the recurrent laryngeal nerve supply to the internal laryngeal muscles is strictly unilateral this bilateral response cannot be explained as an effect of motor fibre stimulation. It is possible that sensory fibres in the recurrent laryngeal nerve are stimulated. The impulse reaches the central motor nuclei and elicits a reflex motor contraction on both sides similar to that which takes place in the cough reflex. There is direct and indirect supportive evidence to this assumption in the works of Eyzaguirre et al. (1966) and Suzuki & Kirchner (1969)

Superior laryngeal nerve stimulation produced adduction of the vocal fold to a less median plane than did stimulation of the recurrent laryngeal nerve. Such an effect was observed only in cases where the external

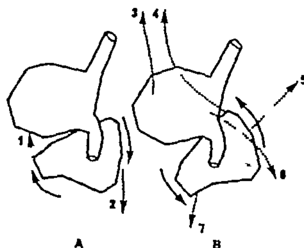


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CLINICAL APPLICATION OF ELECTROMYOGRAPHY IN VOCAL FOLD MOBILITY DISORDERS

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Abstract Some salient problems concerning the diagnosis and prognosis of vocal fold mobility disturbances are briefly outlined. The application of laryngeal electromyography in such cases is recommended. A brief review of EMG findings in the normal and diseased neuro-muscular system is given. In the present study electromyographic investigations of the laryngeal muscles were made of 13 patients (5 males and 8 females) with ages ranging from 22 to 70 years. Their clinical diagnoses included a variety of etiological factors. The posterior crico-arytenoid, thyro-arytenoid and crico-thyroid muscles were routinely examined. A method for the performance of these tests, in the form of an initial sitting and repeated follow-up control studies, is given. The clinical versus EMG findings are analysed. The criteria of neuropathic changes in laryngeal electromyograms are described and their significance is discussed.

It is customary to designate a case of vocal fold immobility as "cordal paralysis" The latter term, however is not necessarily a justifiable conclusion. There is always a question in which of these cases the cordal immobility is due to a neuro-muscular lesion, in contrast to mechanical fixation and functional involvement. Staging of the clinical condition, as well as the follow-up of the behaviour of the vocal fold, has been greatly influenced by the teachings of Sir Felix Semon (1881) Terms such as abductor paralysis, adductor paralysis, partial or complete recurrent nerve affection have become standard expressions to describe clinical conditions Much work has been devoted to provide a physio-anatomical explanation for the cited observations Some investigations, however have pointed to the non-reliability of the Semon doctrines. The inconsistency of the descriptive terminology of vocal fold positions, as seen on laryngoscopy has added to the difficulty of providing an agreement concerning the categorization of the immobile vocal fold. This has already given rise to apparently radical differences of opinion among the pioneers in the field, such as with Rosenbach (1880) and Semon (1881) on the one hand, and Wagner (1890) and Grossmann (1897) on the other

Faced with these diagnostic and prognostic problems, we felt, in agreement with earlier investigators (Feinstein, 1945–6 Faaborg-Andersen, 1957 Kotby 1967 Hiroto et al., 1968) the necessity of applying electromyography as a clinical diagnostic aid to reveal the neuromuscular status of the various laryngeal muscles. Electromyographic findings can point to the site and type of lesion in the neuro-muscular system and provide the basis for follow up studies In clinical electromyography potential changes occurring across the muscle cell membrane are recorded extracellularly by needle electrodes inserted into the muscle Under normal conditions a properly relaxed skeletal muscle is electrically silent. On contraction there is a series of potential changes occurring across the cell membrane These are recorded as the motor unit action potentials (MUAP), which are usually biphasic or triphasic The

duration of the MUAP as recorded by a monopolar needle electrode, ranges between 2 and 10 msec, and the amplitude between 100 and 2 000 µV (Rodriguez & Oester 1961) The discharge rate is 5–50 spike/sec (Buchthal, 1957) The highest frequency of MUAP discharge occurs on maximal contraction. This is called full interference pattern. Neurogenic disturbances, which imply any lesion affecting the motor unit, from the motor neuron cell body to the nerve terminals, will give rise to a certain denervation activity At rest the muscle may show various types of spontaneous electrical activity These are in the form of brief (0.5–3 msec) potentials, usually biphasic, of very small amplitude (50–200 µV) designated as fibrillation potentials, or monophasic positive potentials known as the positive denervation potentials. In central lesions (anterior horn cell lesions) high voltage polyphasic spontaneous activity fasciculation potentials, are recorded. The MUAP recorded on attempted contraction are usually broad. The number of activated motor units is reduced to a varying extent even on maximal contraction. Consequent on reinnervation the incidence of denervation activity decreases. Polyphasic potentials, usually of low amplitude, appear during voluntary contraction. These are usually called regeneration potentials If the muscle fibre is the site of the insult there will be characteristic changes in electrical activity usually described as myopathic changes. The MUAP are usually shorter in duration with frequent polyphasic potentials. There is full interference pattern, with firing of most of the surviving motor units in response even to a mild degree of contraction. Spontaneous fibrillation potentials may occur in myopathies of long standing.

MATERIAL AND METHOD

The present series of investigations of patients with immobile vocal folds comprises 13 cases. 5 males and 8 females. Their age ranges between 22 and 70 years. All were referred with the diagnosis of either vocal cord paresis or recurrent nerve paralysis, sometimes together with other cranial nerve involvement. Clinical, laryngological and neurological, as well as radiological investigations yielded a variety of etiological factors (Table I). The posterior crico-arytenoid, thyro-arytenoid and crico-thyroid were the muscles routinely examined. In the first sitting the posterior crico-arytenoid muscle was examined by direct approach. This test was carried out in a shielded endoscopy theatre under neurolept analgesia. This is a type of analgesia in which the apprehension of pain is alleviated to a considerable degree, while consciousness is not seriously hampered. In this way the patient can respond to instructions given by the operator The patients of the present series received Dropendol intramuscularly (0 10–0 15 mg/kg) 1 hour before the examination. On the arrival at the theathre the patients received Phentanyl intravenously (0 05–0 1 mg) Neurolept analgesia was supplemented by topical pharyngo-laryngeal analgesia, using Lidocain spray The larynx was then explored by a Magill laryngoscope, and concentric needle electrodes (DISA 13K51) were inserted into the posterior crico-arytenoid muscle under direct vision. The other laryngeal muscles tested, the thyro-arytenoid and crico-thyroid, were examined by the percutaneous technique. The details of this technique have been described by Kotby (1967) For follow-up studies however only the percutaneous technique was applied, which does not necessitate the administration of any sort of analgesia. The MUAP were recorded by a DISA three-channel electromyograph with amplifier 13A84 The camera device allows a continuous recording with a speed of 5 cm/sec and an interrupted recording with 1 mm/msec.

EMG registrations were carried out while the patient was attempting to perform various laryngeal actions, respiration, phonation and sphincteric actions. It was possible to study tracings from 1–2 recording points only In fact, there is little manoeuverability in electrode adjustment, because of the small dimensions of the laryngeal muscles. The parameters

Table I

Case	Clinical diagnosis	Duration	VC position	EMG Spontaneous activity	Parameters of MUAP	Interference pattern
1	Postop. Lt. RLN palsy (Operated coarctation of the aorta)	2 months	Paramed.	Nil	Broad (5–7 msec)	Mixed
2	Rt. RLN palsy	3 months	Paramed.	Nil	Normal (2–4 msec)	Full
3	Lt. RLN palsy	4 years	Paramed.	Nil	Broad (5 msec)	Mixed
4	Lt. IX–X–XI cranial nerve palsy	9 months	Paramed.	Nil	Broad (4–7 msec)	Mixed
5	Post-traumatic paresis of both VC	1 month	Aberrant (hematoma)	Nil	Normal	Full
6	Post-thyroidectomy bilat. RLN palsy	14 years	Bilat. paramed.	Nil	Normal	Full
7	Post-thyroidectomy Rt. RLN palsy	2 months	Paramed.	Nil	Normal	Fair mixed
8	Bilat. paralysis of the VC	3 months	Intermed. (variable)	Nil	Normal	Full
9	Rt. VC paralysis, Metastatic carcinoma of the breast	5 years (?)	Paramed.	Nil	Normal	Full
10	Lt. RLN palsy· Lt. hemithyroidectomy· Aortic arch syndrome	13 years	Paramed.	Nil	Broad (6–7 msec)	Fair mixed
11	Paralysis of Lt. VC, Lt. parapharyngeal tumour	8 months	Intermed.	Present	Broad (5–7 msec)	Mixed
12	Bilat. paralysis of VC (Functional)	1½ months	Intermed.	Present	Broad (7 msec)	Discrete
13	Post-thyroidectomy bilat. RLN palsy	4½ years	Paramed.	Nil	Broad (5–7 msec)	Discrete

The clinical and electromyographical findings in the present series of patients. RLN Recurrent laryngeal nerve. VC vocal cord. PCA. Posterior crico-arytenoid. Th. A. Thyro-arytenoid. Cr Th. Crico-thyroid. Conf Confirmed. Comb. Combined. Rt. Right. Lt. Left.

of the MUAP were analysed from the recorded respiratory activity During the respiratory fluctuation electrical activity is minimal, thus allowing the detection and analysis of individual MUAP The duration of the MUAP was considered fundamental for the evaluation of the diagnosis. The tracings were further analysed by studying the interference pattern on attempted maximal contraction, as during straining and phonation.

RESULTS

The results obtained from the study of the present series are illustrated in Table I The electromyographic findings did not confirm the clinical diagnosis in 6 cases out of 13 In 4 cases (case 2, 6 8 and 9) with the diagnosis of recurrent nerve paralysis the neuro-muscular system proved to be electrically normal. In case 12 the clinical diagnosis was in favour of

Muscles examined	Muscles affected	Comments
Bilat. Th. A. Bilat. Cr Th.	Lt. Th. A. Lt. Cr Th.	Comb. neurological affection
Bilat. PCA Bilat. Th. A.	—	EMG-normal neuromuscular system
Bilat. Th. A. Bilat. Cr Th.	Lt. Th. A. Lt. Cr Th.	Comb. neurological affection
Bilat. PCA Bilat. Th. A.	Lt. PCA	Conf. Lt. RLN palsy
Bilat. PCA Bilat. Th. A.	—	EMG-normal neuromuscular system
Lt. Th. A. Bilat. Cr Th.	—	EMG-normal neuromuscular system
Bilat. PCA Lt. Th. A.	Rt. PCA.	Conf neuropathic lesion of Rt. RLN
Bilat. Th. A. Bilat. Cr Th.	—	EMG-normal neuromuscular system
Bilat. PCA. Bilat. Th. A Bilat. Cr Th.	—	EMG-normal neuromuscular system
Bilat. PCA	Lt. PCA	Conf neuropathic lesion of Lt. RLN
Bilat. PCA Lt. Th. A. Lt. Cr Th.	Lt. PCA	Conf neuropathic lesion of Lt. RLN
Lt. PCA Bilat. Th. A. Bilat. Cr Th.	Lt. PCA Bilat. Th. A. Bilat. Cr Th.	Bilat. comb. neurological affection
Lt. PCA Lt. Th. A.	Lt. PCA	Conf neuropathic lesion of Lt. RLN

a functional basis for vocal cord immobility Electromyography however revealed in this case a severe neuropathic lesion. In Case 5 of suspected disruption of the muscles of the vocal fold following laryngeal trauma, electromyographic study showed normal findings. In addition, in Cases 1 and 3 the diagnosis was extended to include involvement of the external laryngeal nerve.

The most constant electromyographic change in cases with neuropathic lesions was the lower number of activated motor units on attempted maximal contraction, giving rise to a mixed to discrete pattern (Fig. 1) In 7 cases, only very few 2–4 MUAP were recorded. In Cases 4 and 12 we were able to trace only 1 MUAP (Fig. 2) Comparing the figures obtained for the duration of these MUAP however, with the duration–distribution curve for normal laryngeal MUAP (Fig. 3), it is seen that they either fall on the extreme maximal normal limits or have a value greater than the normal figures.

In a few cases (2 out of 8) brief biphasic potentials of low amplitude were recorded (Fig. 4). These were interpreted as fibrillation potentials. They were evaluated retrospectively by studying the whole record It is usually difficult to identify fibrillations amidst the basic electrical activity of the laryngeal muscles, in contrast to the fully relaxed peripheral skeletal muscles. These brief potentials, when encountered, were considered diagnostic of a neuropathic lesion.

DISCUSSION

Previous investigations have generally demonstrated that neuropathic laryngeal electromyograms show the main changes encountered with in peripheral skeletal muscles (Feinstein 1945–6 Macbeth, 1945–6 Faaborg-Andersen, 1957 Greiner et al 1960 Kotby 1967 Haguenauer et al., 1967 Hiroto et al., 1968) A special stress has been made upon the identification of fibrillation potentials. However Faaborg-Andersen (1957) and Kotby (1967) have pointed out the difficulty in detecting fibrillation potentials in laryngeal neuropathic electromyograms. Normal laryngeal MUAP are of brief duration and low amplitude and may be

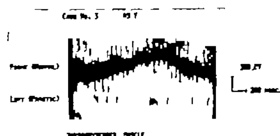
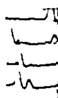


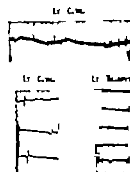
Fig 1 There is marked reduction of activated MUAP on the paretic side as compared with the normal side.





MOTOR UNIT ACTION POTENTIALS IN PARETIC VOCAL FOLDS

Fig 2 Few MUAP were recorded from the paretic laryngeal muscles. These were considerably broader than the normal laryngeal MUAP



BRIEF "FIBRILLATION POTENTIALS"

Fig 4 Many brief biphasic fibrillation potentials could be recorded in this case. A suspected positive denervation potential can be seen in the record from the left thyro-arytenoid muscle (*Lt. Th. Aryt.*).

misinterpreted as fibrillation potentials. They also stressed that in paretic vocal cords, electrical activity with phonation and sphincteric actions of the larynx was less in amplitude than on the normal side.

In the present study the laryngeal neuromuscular system presented certain peculiarities concerning the interpretation of the electromyographic findings The most constant finding in neuropathic laryngeal electromyograms was the reduction of the number of motor units on activation. Such a reduction *per se* does not indicate a neuropathic lesion in peripheral skeletal muscles It is encountered within these muscles under normal conditions when the muscle is under a moderate degree of tension. In laryngeal movements, however there is little chance for gradation of the degree of contraction. The muscles of both sides of the larynx are normally activated to almost the same degree in actions like straining, phonation or respiration (Kotby 1967) Accordingly the de

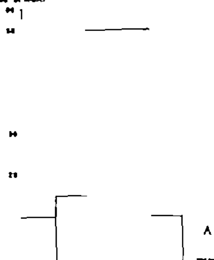




Fig 3 (*A*) Histogram illustrating the mean duration of the normal MUAP recorded from the thyroarytenoid muscle as constructed from the analysis of 145 individual MUAP (*B*) Histogram illustrating the percentage distribution of MUAP duration. Paretic histogram (*hatched*) is constructed by the analysis of 15 individual MUAP only due to the scarcity of the recorded MUAP from each paretic muscle. (Histogram for normal MUAP duration modified from Kotby 1967.)

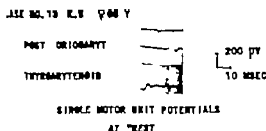


Fig 3 Individual motor units recorded from the posterior crico-arytenoid muscle (*upper trace*) and the thyro-arytenoid muscle (*lower trace*), demonstrating that the electrodes are picking up activity from different motor units in these two muscles.

tection of a significant difference in the interference pattern in identical muscles on both sides of the larynx can be taken as pointing to a neuropathic lesion of the muscle.

The second most significant criterion for laryngeal neuropathic lesions was the broadening of the MUAP This, however should be considered critically since it is only a relative increase of the mean duration (Fig. 3) The detection in laryngeal electromyograms of MUAP above 4 msec in duration should be taken as suggestive of a neuropathic lesion

The aforementioned changes are usually considered to be suggestive of denervation in peripheral skeletal muscles. Fibrillation potentials, however, are regarded as of a greater diagnostic value, while positive denervation potentials are considered pathognomonic of denervation. In small internal laryngeal muscles a state of "rest" with electrical silence is unattainable. There is always a certain degree of resting tonic activity in the form of a low frequency discharge of MUAP of brief duration and low amplitude. The lack of an electrically silent background makes the identification of fibrillation potentials extremely difficult by the usual audio-visual appliances of the electromyograph. In the present series brief potentials of particularly low amplitude were, nevertheless, found in 2 cases (Cases 11 and 12), amidst the resting activity on studying the recorded tracing. These potentials were taken as fibrillation potentials Their significance as being diagnostic of a denervation process is maintained. In a single case (Case 12) of a severe neuropathic lesion, a suspected positive denervation potential was recorded (Fig. 4) Though rarely seen, these denervation potentials are considered pathognomonic of a denervation process.

From the preceding it is evident that though neuropathic data are essentially the same for small internal laryngeal muscles as for peripheral skeletal muscles, the diagnostic importance of each of these changes is in some respect peculiar to the larynx.

The absence of abnormal electromyographic manifestations in 5 cases where the clinical diagnosis was pointing to a neuro-muscular involvement, necessitates critical evaluation. It has been claimed by Dedo & Hall (1969) that EMG-recordings of the small internal laryngeal muscles made with concentric needle electrodes with lead-off surface of 0 015 and 0 07 mm² are not completely reliable. They suggest that these electrodes pick up a significant amount of cross talk potentials from nearby muscles over 0.5 cm distant from the recording electrode. Thus an electrode inserted into a paretic thyro-arytenoid muscle can pick up activity from the normal crico-thyroid muscle, in addition to the thyro-arytenoid. Our experiments with concentric needle electrodes, DISA 13K51 with a lead-off surface of 0 07 mm showed, however that simultaneous recording from the thyro-arytenoid and the ipsilateral posterior crico-arytenoid muscles failed to demonstrate synchronization of the MUAP recorded from these two muscles (Fig. 5)

Hiroto et al. (1968) suggest that the detection of normal electrical activity in laryngeal muscles in cases of immobile vocal cords can be explained on the basis of misdirection of regenerating nerve fibres. "When the regenerating nerve fibres from the central segments that have primarily innervated adductor muscles reach not only the adductor but also the abductor muscles, these antagonists contract simultaneously and result in cancellation of their effects

The assumption of misdirected innervation

Table II

Case	Neuro-laryngological status	Follow-up EMG	Follow-up Clinical
1	Lt. sided combined motor neurological affection	Regeneration	Improved
2	Electromyographically normal neuromuscular system	————	Unchanged
3	Lt. sided combined motor neurological affection	Unchanged	Progress to other cranial nerves
4	Lt. recurrent laryngeal nerve palsy	Regeneration	Improved
5	Electromyographically normal neuromuscular system	————	Improved
6	Electromyographically normal neuromuscular system	————	Unchanged
7	Rt. recurrent laryngeal nerve palsy	Regeneration	Improved
8	Electromyographically normal neuromuscular system	————	Improved
9	Electromyographically normal neuromuscular system	————	Unchanged
10	Lt. recurrent laryngeal nerve palsy	Unchanged	Unchanged
11	Lt. recurrent laryngeal nerve palsy	Unchanged	Unchanged
12	Bilat. combined motor neurological affection	Regeneration	Improved
13	Bilat. recurrent laryngeal nerve palsy	Unchanged	Unchanged

Follow-up observations of the cases submitted to EMG investigation were carried out up to 1 year

cannot explain all aspects of the problem. The muscle or muscle group receiving misdirected regenerating fibres largely retains its original nerve supply as well Tension produced in response to a volley of impulses coming via the misdirected fibres is expected to be less than that produced under the influence of the original innervation. Accordingly such a weak contraction is not expected to disturb significantly the state of equilibrium in the system of forces acting on the crico-arytenoid joint. Moreover there is still a question to be raised hether the posterior crico-arytenoid muscle on the one hand and all the other small internal aryngeal muscles on the other hand are to be considered true antagonists

It is believed that in the case of nerve palsy the number of active motor units may vary considerably in the different groups of muscles. Thus there is a great deal of gradation in the degree of muscular failure, rather than a sharp abduction or adduction failure In the light of this assumption abnormal EMG-findings are expected to be detected in various small internal laryngeal muscles, irrespective of the position adopted by the immobile vocal cord (Bonner & Atkins, 1968) In Cases 1 and 3 *the immobile vocal fold was in the paramedian* position, indicating an abductor paralysis" Though the abductor" the posterior crico-arytenoid muscle, was not examined, the detection of abnormal EMG-findings in two of the members of the adductor group may indicate a widespread involvement rather than an isolated posterior crico-arytenoid muscle affection.

In 6 out of the 13 cases the EMG-findings were shown to contradict the clinical diagnosis. In 5 cases the EMG-findings excluded a neuropathic lesion, which had been suggested on clinical ground. In one of these (Case 8) a functional basis was strongly suggestive The position of the vocal folds was changing at different *examinations. On the other hand,* Case 12 was diagnosed clinically as a functional disorder This assumption was based mainly on the inability to categorize the behaviour of the vocal folds according to conventional teaching to fit with any specific neurogenic lesion. EMG study showed data highly suggestive of a severe neuropathic lesion, involving the recurrent laryngeal and the external laryngeal nerves on both sides, with abundance of fibrillation potentials The muscles tested were almost electrically silent on attempted voluntary contraction. The etiological background was suggested to be a toxi-infective factor The patient had suffered from a severe upper respiratory in-

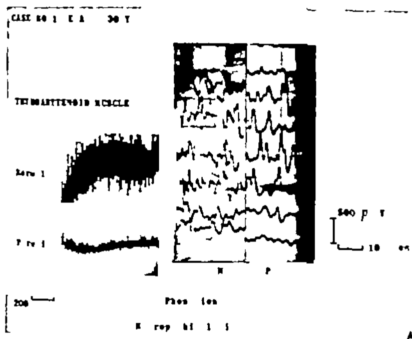
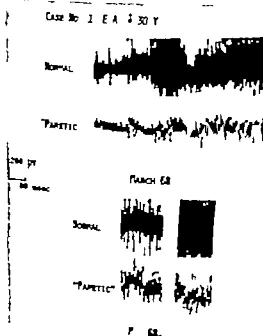


Fig. 6 (A, B) Follow-up recordings from the thyro-arytenoid muscle on both sides. Notice the paretic (*left*) side recovering to almost normal degree of electrical activity after 3 months.



fection shortly before the onset of the vocal disability. In Case 5 vocal cord mobility disturbance was attributed to mechanical interference by a huge submucous hematoma. Vocal cord mobility returned to normal after resorbtion of the hematoma.

The normal EMG-findings in Case 2 led to the suggestion that the mobility disturbance was probably due to articular ankylosis. Clinical follow-up examination over a period of 4 months showed no change in the behaviour of the vocal fold. In Case 9, with report of a long-standing vocal cord immobility, there was a history of mediastinal metastases, which might have involved the recurrent laryngeal nerve. The detection of normal EMG-findings at the present stage of the disease may be explained by regeneration of the nerve fibres after regression of the metastases under hormonal therapy. The cordal immobility may be maintained by an articular ankylosis that has developed during the period of immobility when the nerve was affected. The same explanation of articular ankylosis developing during the immobile phase can be applied to Case 6. Nerve regeneration at a later stage can explain the normal EMG-findings.

Table II, which summarizes the results of the follow-up studies of the investigated cases, reflects certain aspects of the conflict of EMG versus laryngoscopy. The importance of EMG investigations for follow-up evaluation is illustrated in Case 1, where signs of reinnervation

could be detected electromyographically long before any clinical improvement of the mobility disturbance was noticed (Fig. 6) In cases where EMG shows neuropathic manifestations the prognosis can be traced directly by studying further changes in the electrical activity of the muscles involved. The prognosis of cases with immobile vocal folds and normal EMG activity is more variable The ultimate outcome of the vocal fold mobility will depend on the primary etiological factor. It is stressed, however that this neuroph[illegible]gical investigation should be considered a [illegible]entary test.

CONCLUSIONS

1 Electromyographi[illegible] [illegible]igation of laryngeal muscles has a well [illegible] place in the diagnostic and prognostic [illegible]uation of cases with vocal fold mobility disturbances The essence of the test lies in its reputation for comparative and follow-up studies It can help to differentiate mechanical and functional factors from neuro-muscular lesions producing vocal fold mobility disorders EMG investigations can also indicate whether the recurrent laryngeal nerve or the external laryngeal nerve alone is involved, or whether there is a combined motor involvement.

2 It is rec[illegible]nended that in the first sitting the test is carried out in the endoscopy theatre under neurolept analgesia. The posterior crico-arytenoid muscle is tested by direct electromyographic recording *per vias naturales* The rest of the muscles are tested by the percutaneous technique. For follow-up examinations the percutaneous method affords a satisfactory tool and can be performed without anaesthesia in the EMG clinic The number and frequency of follow-up investigations should be arranged for each individual case according to its own merits.

3 The criteria of neuropathic lesions of the laryngeal neuromuscular system are in the order of frequency (*a*) reduction of the number of MUAP in the form of a discrete or a mixed pattern. (*b*) broadening of the MUAP (*c*) fibrillation potentials, though rarely recorded, are highly diagnostic These have to be searched for carefully in the record and the resting electrical activity of the muscle.

ZUSAMMENFASSUNG

Einige Hauptprobleme betreffend der Diagnose und Prognose der Störungen von Stimmfaltenbewegung werden kurz besprochen. Die Anwendung von Elektromyographie am Kehlkopf wird in solchen Fällen empfohlen. Ein kurzer Überblick über die EMG-Befunde im normalen und kranken neuromuskulären System wird gegeben. In diesem Studium wurden elektromyographische Untersuchungen der Kehlkopfmuskeln an 13 Patienten (5 Männern und 8 Frauen) im Alter von 22 bis 70 Jahren angewandt. Ihre klinischen Diagnosen umfassten viele verschiedene ätiologische Faktoren. Der M crico-arytenoideus post., thyreo-arytenoideus und crico-thyreoideus wurden routinemässig untersucht. Eine Methode für die Ausführung dieser Teste, in Form von einer Initialuntersuchung und wiederholten „follow up Kontrollen, wird angegeben. Die klinischen Resultate werden mit den EMG Befunden zusammengestellt und analysiert. Die Kriterien der neuropathischen Veränderungen in den laryngalen Elektromyogrammen werden beschrieben, und ihre Bedeutung wird diskutiert.

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TRACHEAL DYSTONIA AND SARCOIDOSIS

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Abstract A case of tracheal dystonia is submitted. In a 44-year-old woman with increasing dyspnoea of predominantly expiratory type, bronchoscopy revealed abnormal yielding and bulging of the right side of the membranous tracheal wall. The bulging was situated in the upper 8 cm of the trachea and varied in extent with the intratracheal pressure. At operation the membranous wall was found to be extremely yielding, in spite of normal width. Adjacent to the abnormal part of the membranous wall there was an enlarged lymph node which on microscopic examination exhibited changes indicating sarcoidosis. The membranous wall was supported by a strip of Teflon, and since the operation the expiratory dyspnoea has improved. The present case is compared with reported cases of tracheobronchial dystonia. It is concluded that the yielding of the membranous wall may have been due to long-lasting pressure by the lymph node destroying the submucous elastic fibres.

…heobronchial dystonia (tracheobronchial …llapse, dyskinesie tracheobronchique) is characterized by expiratory stenosis of the trachea and large bronchi caused by abnormal yielding of the membranous wall. This phenomenon was first described by Lemoine in 1949 later by int. al. Garaix (1952) and Herzog & Nissen (1954) In Scandinavia the first case was reported by Ehrner (1954)

A definition of the disease is somewhat difficult, since the degree of normal narrowing of the lumen of the trachea and large bronchi during expiration and coughing has not been finally elucidated in spite of numerous investigations. Campbell (1967) quoted several authors who found, by bronchoscopy and X ray cinematography a narrowing of the lumen of about 50% at rest. Hodges et al (1966), by video tape recording, found a narrowing of the lumen of more than 50% in over 60% of the patients in a non-selected series.

Tracheobronchial dystonia is most common in men over 50 years of age. In 12 745 bronchoscopies Vaselin et al. (1966) found 54 cases, but only 4 were so severe as to call for surgical treatment. Herzog (1958) found 16 cases in 1 500 bronchoscopies. Among 1 130 patients with pulmonary tuberculosis Donno et al. (1954) found 21 cases.

The aetiology is unknown. The abnormally yielding membranous wall is observed most often in patients with chronic bronchitis and bronchial asthma. Herzog (1958) therefore, believes that the submucous elastic fibrils are destroyed by long-lasting strain due to coughing and submucous inflammation. In Campbell's (1967) opinion the primary cause in some cases is a constitutional variant in tracheal shape (the so-called lunate shape) with a very broad membranous wall. In these cases the membranous wall makes up a relatively large part of the tracheal circumference, and this leads to reduced tracheal stability

The pathophysiology has been described by Herzog (1958) who stated that the changes in tracheal lumen which occur synchronously with the respiration are determined by the stability of the tracheal wall and by the dif

ference between the extra- and intra-tracheal pressure (the transmural pressure) During vigorous coughing the intrathoracic pressure may increase up to 150 mm Hg. When the flow resistance in the small bronchi is increased, the transmural pressure increases markedly and if the membranous wall is at the same time abnormally yielding, the tracheal lumen may become totally occluded during coughing. This valvular stenosis starts a vicious circle, of insufficient cough and stagnation of secretion.

The severity of the symptoms varies from case to case, from mild expiratory dyspnoea to severe attacks of expiratory tracheal stridor which may lead to asphyxia. During the attacks, which are provoked by coughing and physical exertion, the patients, exhibit severe agitation and anxiety irritative cough, a sensation of a bolus, and dysphagia. Auscultation of the lungs reveals an extended chest with small respiratory excursions. A few rales may be heard, but the auscultation is predominated by the expiratory stridor

The diagnosis is confirmed by bronchoscopy At rest and at quiet respiration the membranous wall is broad, smooth, without longitudinal folds. During inspiration the lumen of the trachea and large bronchi will be increased, the membranous wall being displaced backward. During expiration, and especially during forced expiration and coughing, the membranous wall bulges forward reducing the lumen to a semilunar slit or in some cases occluding it entirely This is most commonly encountered in the lower third of the trachea and upper part of the main bronchi. The cartilaginous wall is of normal stability

Other diagnostic procedures are bronchography with cinematographic exposures or videotape recording (Hodges et al., 1966) Among pulmonary function tests that of forced expiratory volume is of importance, as Tiffeneau s curve may show kinking at an early stage of expiration, indicating valvular stenosis. Direct measurements of the pressure in the trachea and bronchi with simultaneous recording of the intra-oesophageal pressure, changes in volume and flow rate (H rzog & R t 96. Campbell & Faulks, 1959) afford r t ty of fairly accurate determinations of t. ,w resistance in the various tracheo-br g-ments.

The results of the named studi a m portance in deciding whether or n t patient is a candidate for surgery Th urgical procedure has been described by H r r & Nissen (1954) who supported the l w p t of the membranous wall by a bon/ pl Later Rainer et al. (1968) and others h e mployed polyethylene and Dacron-r inf r e t Silastic So far more than 200 patients have be n treated surgically but the material ext em l heterogeneous, as in many cases the pr d re has been done in connect.on with major int n thoracic operations (lobectomy neum m tomy) As a rule, the primary effe able. The asphyctic attacks disappear it exertional dyspnoea as well as the ex tora tion decrease However relapse ha e r ported in some cases. Rainer et al. 1 68) has published 2 cases with a fatal outcome because of erosion of neighbouring organs, caused by the implanted material.

In patients not suited for surgical treatment a good effect may be obtained by conservative measures directed at co-existing bronchial asthma or chronic bronchitis. This counteracts the valvular stenosis by reducing th resistance in the small bronchi.

The following case history is probably of interest because of the unusual localization of the expiratory stenosis and because the disease does not appear to have been reported previously in association with sarcoidosis.

Case Report

A 44-year-old housewife, who had previously been in good health, had been suffering for the past 4 years from increasing respiratory embarrassment. During the first 3 years or so she was bothered by expiratory dyspnoea only on physical exertion. During the past year however there had been audible inspiration and expiration also at rest, especially in

zontal position and mainly when she was lying on her left side She had a dry cough, and during the coughing attacks there would be increasing expiratory distress with a feeling as if she were about to vomit.

Clinical examination showed no enlarged lymph nodes on the neck, but a suggestion of venous congestion. Auscultation of the lungs revealed scattered rales and expiratory whistling over both posterior lung surfaces and in both axillae. The *whistling was also audible at a* distance during oral respiration. Indirect laryngoscopy X rays of the trachea, chest, and oesophagus with contrast medium were normal. Scanning over the thyroid gland and the basal metabolic rate were normal. Tomography of the superior mediastinum showed a slight increase in width with a suggestion of a polycyclic definition of the right contour Pulmonary function tests were normal, the forced expiratory volume being 70% of the vital capacity

Bronchoscopy under general anaesthesia disclosed on the right side of the membranous wall a 8 cm bulge beginning about 2 cm below the glottis, increasing for the first 3 cm, and thereafter gradually decreasing towards the carina The bulge was smooth, non-pulsating, soft, and compressible The mucosa of the membranous wall was smooth, without longi-al folds The cartilaginous wall was of al appearance and stability *The bulge* disappeared almost completely during the ventilation, but immediately became reproduced, reducing the tracheal lumen by about 30% Towards the end of the investigation, when the patient was made to strain, the lumen was reduced by 60–70% Microscopic examination of a mucosal biopsy from the most prominent part of the bulge showed no abnormality

The patient was transferred to a department of thoracic surgery[1] where right-sided thoracotomy was performed owing to a suspicion of a benign mediastinal tumour At operation the lungs, mediastinum, and the cartilaginous wall of the trachea were found to be normal. In spite of a normal width, the membranous wall was extremely weak. In the angle between the trachea and oesophagus, on a level with the 1st and 2nd tracheal rings, there was a soft, enlarged lymph node measuring 2 × 1 × 1 cm. Microscopic examination of the excised lymph node showed epithelioid-cell granulomas as in *sarcoidosis Ziehl-Nielsen staining showed no* acid-fast rods. The entire membranous wall was supported by a Teflon strip sutured laterally to the cartilage rings The postoperative course was uneventful, and at follow-up the patient showed marked improvement of the exertional dyspnoea, the cough had subsided, and auscultation of the lungs was normal.

DISCUSSION

Tracheal stenoses may be divided into (*a*) Obturations, caused by foreign bodies or pedunculated lesions arising in the tracheal wall (*b*) Organic stenoses whose cause is localized peritracheally (compression stenoses) or within the tracheal wall proper (*c*) Functional stenoses which may be divided into hypertonic (spastic) and hypotonic. The hypotonic functional stenoses may be due to abnormal yielding of the cartilaginous wall (tracheo-malacia) or the membranous wall (tracheal dystonia)

Boucher et al. (1959) have subdivided tracheobronchial dystonia, by presumed aetiology and pathogenesis, into primary and secondary by the extent of the lesion into diffuse or localized, and by the course into irreversible and transient.

Considering the variations in extra and intra-tracheal pressure synchronous with the respiration, intrathoracic stenoses will be expected to give rise to expiratory stridor extra thoracic stenoses to inspiratory stridor The explanation why the present patient had predominantly expiratory distress, in spite of the partially extrathoracic site of the stenosis, was

[1] Thanks are due to Jens L. Hansen, M.D., Head of the Department of Thoracic Surgery Bispebjerg Hospital, for the permission to use the case records.

presumably that the variations in intrathoracic pressure are transmitted up behind the auxiliary respiratory muscles of the neck.

The patient had an enlarged lymph node adjacent to the abnormally limp and bulging membranous wall. It is unlikely that there could be a combination of two independent diseases, as the localization is highly suggestive of a relationship. However the disproportion between the size of the node and the extent of the bulging indicates that apart from a certain compression, a structural change has taken place in the membranous wall. The bulging in tracheobronchial dystonia is usually symmetrical and most marked in the lower third of the trachea. However Herzog & Nissen (1954) has described cases in which the maximum bulging was in the middle third, and Diané et al. (1966), among others, have described asymmetrical bulging, most often on the right.

Compression of the bronchi is not uncommonly encountered in sarcoidosis. Compression of the main and segmental bronchi has been reported by Grimminger (1955) and by Kämpfer (1959) and others.

In our case the abnormal yielding must be assumed to have been caused by destruction of the elastic elements of the membranous wall, due to organic changes or chronic pressure by the enlarged lymph node.

According to Boucher's classification, then, the present case was one of secondary localized tracheobronchial dystonia.

ZUSAMMENFASSUNG

Ein Fall von Dystonia Tracheae wird beschrieben. Bei einer 44jährigen Frau mit zunehmender Dyspnoe wurde bei der Bronchoskopie eine abnorme Erschlaffung und Vorwölbung der rechten Seite der Luftröhrenhinterwand gefunden. Die Lokalisation der Vorwölbung war die superiore 8 cm der Luftröhre. Bei Änderungen des intratrachealen Druckes änderte sich auch die Grösse der Vorwölbung. Bei der Operation hatte die Luftröhrenhinterwand normale Breite aber sie war sehr erschlafft. Hinter dem abnormen Teil der Luftröhrenhinterwand wurde ein grosser Lymfknoten gefunden, der histologisch als Morbus diagnostiziert wurde. Die Luftröhrenhinterwand wurde durch eine Teflonprothese verstärkt, wonach die Symptome sich wesentlich erbesserte. Dieser Fall wird mit früher beschriebenen Fälle [illegible] und es wird konkludiert, dass die abnorme Erschlaffung der Luftröhrenhinterwand durch langdauernde [illegible] wirkung des Lymfknotens mit Destruktion d[illegible] [illegible] mukösen elastischen Fasern verursacht [illegible].

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ON THE MECHANISM OF MODULATING THE VOLUME OF TH[illegible] VOICE IN HOWLING MONKEYS

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Abstract It is proposed that the howling monkeys intensify the low pitched sounds, which are emitted in their howls, by considerably narrowing the laryngeal vestibule in order to build up a pressure capable of distending the collapsed thyrohyoid canal. In this manner the vibrating air column can enter the hyoid bulla and the latter act as a resonator The narrowing of the laryngeal inlet is achieved by a pneumatic sphincter—the pharyngolaryngeal sacs—activated by extralaryngeal muscles.

The howling monkeys (genus *Alouatta*) inhabit an extensive area of the tropical and subtropical forests of Central and South America. They have been known for quite sometime for the very loud roars which they make at certain times of the day (Carpenter 1934 Chivers, 1969 Southwick, 1963) The intensification of the grunt-like, low pitched sounds which are produced by these animals when they emit their very high volume and long sustained howl, presents the following problem among others. It is known that low guttural tones are brought about with the vocal cords relaxed, foreshortened, and separated by a wide gap (Pressman & Kelemen, 1955) Such an arrangement at the glottis is not favorable for increasing infraglottic pressure, and in all likelihood, much less to reach the high intensity of voice which characterizes the howler's howl. Under these conditions the pressure may be, in fact, quite low! It is necessary therefore, to look for means other than an increase in infraglottic pressure to explain the elevated sound intensity which *Alouatta* attains when pr[illegible]duc[illegible]n[illegible] p[illegible]culiar howls

MATERIALS

This investigation is based on the [illegible]et[illegible] d[illegible] section of the entire hyolaryngea[illegible] ap[illegible]atus [illegible] six embalmed, adult red howling m[illegible]ys (*A seniculus*), and on additional obser[illegible]t[illegible]ons of 30–40 μm serial, sagittal sections of th[illegible] organs in three adult females of the same species Similar sections from 6 black howlers (*A caraya*) were also studied (2 fetuses, 1 juvenile female, 1 adult female, 1 juvenile male, 1 adult male)

The anatomical nomenclature used in this paper is that of the *Nomina Anatomica* (1966) except for certain terms which had been previously employed by other authors (see references) and for some new names which I have introduced in order to point out certain specializations of *Alouatta*. These new names are printed in italics

OBSERVATIONS

It is necessary to indicate the modifications of the cartilages and bones of the hyolaryngeal organs, and to explain how these pieces are interconnected, in order to arrive at some [illegible]derstanding of their functioning during

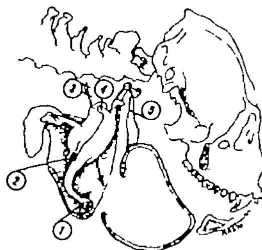


Fig 1 Skeleton of the hyolaryngeal apparatus. The mandible the hyoid bone, and the thyroid cartilage have been sectioned along the midsagittal plane and their respective right halves removed: *1* processus furcatus *2* lingula of cuneiform cartilage; *3* arrow through thyrocuneiform space of left side *4* lingula of epiglottic cartilage *5* cornu branchiale I.



Fig 2 Hyolaryngeal organs sectioned along midsagittal plane, and view of the right half from the midline· *1* intercornual syndesmosis; *2* cartilaginous bridge between cuneiforms; *3* intercuneiform space and cuneiform eminence; *4* vestibular fold, *5* transverse vestibular arch *6* intersaccular chamber· *7* introitus of thyrohyoid canal, *8* black thread passes through the entrance to the lateral air sac, the sac itself (a window opened on the medial wall of the right sac facilitates a view inside this chamber), intersaccular chamber introitus of thyrohyoid canal, the canal, and into the hyodeam sac; *9* hyoepiglottic fat.

tions. The skeletal details are illustrated in Fig. 1 Here the soft parts have been removed to show the laryngeal cartilages and the hyoid Moreover the mandible, the hyoid bulla, the large cup-shaped thyroid cartilage have sectioned along the midsagittal plane and respective right halves removed. The bulla appears as a large and hollowed bone lodged within the mandibular arcade Its wide and semicircular opening faces the epiglottic cartilage The prominent ventral end of the petiolus is bent upward and forked. The two arms of this processus furcatus" (Brandt, 1826 Bernstein, 1923) are connected by syndesmoses to the dorsum of the thyroid laminae, at each side of the midline and near the cranial margin (Figs 2, 3) The enlarged auricle shaped cuneiform cartilages lay parasagittally under the epiglottic blade (Fig. 4) and they are connected to the arytenoids by a syndesmosis. Their dorsal ends are continuous across the midline through a cartilaginous bridge (Fig. 3) Only mucosa and submucosa join the epiglottic and cuneiform cartilages. The submucosa thickens between the *cuneiform lingula* and the petiolus to form the substance of the vestibular folds (Figs. 2, 4) A *thyrocuneiform space* exists at each side between the central cartilaginous complex and the thyroid cartilage (Fig. 1) A large cornu branchiale I (Fig. 3) [see Starck & Schneider 1960 for explanation of this term], articulates with the bulla by a diarthrosis. The dorsal end of this horn is very flexible and it is joined by a distensible syndesmosis to the upper thyroid horn (Figs. 1–3) The tips of both, the cornu branchiale and the superior thyroid horn, are bent medially and embrace the pharynx, thus bringing the intercornual syndesmosis to a position behind the alimentary tube and on level with the laryngeal inlet (Figs. 2, 3) The hyoid bulla and the thyroid cartilage are additionally connected by the thyrohyoid membrane (Figs. 3 4) The latter attaches around the opening of



Fig 3 Craniodorsal view of the hyolaryngeal apparatus. The tongue and the pharynx have been removed, and the laryngeal inlet is clearly seen: *1* bulla hyoidea, *2* thyrohyoid membrane, *3* epiglottis, *4* cuneiform eminence; *5* intercuneiform cartilaginous bridge *6* epiglottic lingula, *7* pharyngolaryngeal sac; *8* intercornual syndesmosis, *9* cornu branchiale I.



Fig 4 Central cartilaginous complex and sacs. Right lateral air sac has been opened, and an artificial window on its medial wall, above the petiolus, connects the two lateral air sacs: *1* epiglottic blade *2* thyrohyoid membrane *3* introitus of thyrohyoid canal *4* processus furcatus, *5* intersaccular chamber *6* transverse vestibular arch; *7* vestibular fold, *8* entrance to left lateral air sac; *9* vocal lip.

the bulla, and along the margins of the upper thyroid horns and laminae The thyrohyoid ligament, whose caudal anchorage is on the laminae, processus furcatus, and on the thyro-epiglottic fibrous joints, is bored by the thyrohyoid canal (Figs 2, 4 5) according to Némai (1926) The cricothyroid ligament is very strong, and the free margins of the cricothyroid membrane form the vocal ligament within the structure called vocal lip (Fig 4) by Kelemen & Sade (1960)

The piriform recesses of the laryngopharynx expand ventrally into the thyrocuneiform space (Fig. 3) as the "pharyngolaryngeal sacs" of Némai (1926) These sacs occupy the rear of the space and are confined between the inferior pharyngeal constrictor laterally and the central cartilaginous complex, more particularly the arytenoids and dorsum of the cuneiforms medially (Fig. 3) Ventrally they are separated from the lateral air sacs by a pad of fatty tissue. The pharyngolaryngeal sacs appear to be constant in *A seniculus* (Némai, 1926 Starck & Schneider 1960 Schön, 1970), *A palliata* (Kelemen & Sade, 1960) and *A caraya* (Böker 193 Schön, 1970)

The inlet of the larynx (Figs. 2, 3) is rather rigid, its contour being formed by the epiglottic cartilage, the dorsum of the cuneiforms, and the intercuneiform cartilaginous bridge. It leads into a vestibule of equally rigid walls, the most prominent features of which are the *cuneiform eminences* (Figs. 2, 3) These paired eminences are quite close near the midsagittal

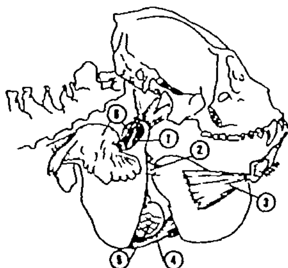


Fig 5 Movement of hyolaryngeal organs: *1 M* middle pharyngeal constrictor 2 cornu branchiale I *3 M* geniohyoideus; *4* thyrhyoid ligament containing the canal; *5* lateral air sac; *6 M* inferior pharyngeal constrictor

line They divide the vestibule into a cranioventral *cuneoepiglottic channel and chamber* and a dorsal more roomy *retrocuneiform channel* (Fig. 2) The vestibular folds are thick, flaccid, and extend from below the cuneiform to the sides of the petiolus, across underside of which the folds are joined a *transverse vestibular arch* (Figs. 2, 4) The folds and the arch form a parabolic arcade the vertex of which appears to rest on the anterior commissure and on its adjacent sides (Fig. 4) The lateral air sacs are large and collapsible expensions of the laryngeal mucosa between the vestibular and vocal folds (Figs. 4 5) These sacs occupy the ventrolateral part of the thyrocuneiform space, and they usually contact each other on the midline above the petiolus. Moreover they are continuous across the midline through an *intersaccular chamber* between the processus furcatus and the dorsum of the thyroid prominence (Figs. 2, 4) Kelemen & Sadé (1960) noted the absence of lateral air sacs in *A palliata*. The thyrohyoid canal is an expansion of the mucosa lining the intersaccular chamber (Figs 2, 4) It penetrates the thyrohyoid ligament (Figs. 2, 4 5) according to Némai (1926) and reaches the hyoid bulla which it lines as the *hyoidean sac* (Fig. 2) The canal is normally curled, and collapsed under the effect of (1) the atmospheric pressure, (2) the tonus of adjacent muscles, and (3) the pressure exerted by the semifluid adipose mass which pads the structures contained in the thyrocuneiform and hyoepiglottic spaces.

DISCUSSION

Previous studies (Kelemen & Sadé, 1960 Némai, 1926) have pointed out the absence of muscular fibres around the inlet of the larynx, the walls of the vestibule, and within the vestibular folds. Moreover none of the extrinsic or intrinsic muscles of the hyolaryngeal organs (Schön, 1964 1968) appear to have any direct effect in modifying the lumen of the inlet and the vestibule or the position of the false vocal cords. However the inlet and the vestibule can perhaps be narrowed by the following mechanism. Presumably the entire larynx is elevated by the pull of the combined insertion of the stylo- and palato-pharyngeus (Schön, 1968) on the posterior margins and lesser horns of the thyroid cartilage (Fig. 5) The superior horns move upward superficially to the hyoid cornua, and the flexible tips of the latter will bend upward and medially In the meantime, contraction of the geniohyoideus (Schön, 1968) will tilt the hyoid bulla so that its base moves down and forward (Schön, 1964), and action in which the sternohyoideus probably participates also. The reciprocal displacement of the thyroid horns and the hyoid cornua is allowed by (1) the flexibility of the cornua branchialia I, (2) the activity of the pars cornualis of the middle pharyngeal constrictor (Schön, 1968), and (3) the mobility of the hyocornual diarthrosis.

The final result of all these movements will be the complete isolation of the pharyngolaryngeal sacs from the rest of the upper respiratory airway because in their new position, the cornua branchialia I will press against the dorsum of the central cartilagnous complex below the laryngeal inlet, and therefore, con-

strict the pharyngeal tube (Fig. 5). Air can thus be trapped in the pharyngolaryngeal sacs, and if pressurized by the activation of the inferior pharyngeal constrictors, it will displace the cuneiform cartilages toward the midline. The lumen of the laryngeal vestibule and inlet will be now narrowed to a further extent. Exhaled air, which has been activated at the glottis, will find an additional obstacle in leaving the larynx, and pressure will build below the obstacle in the supraglottic part of the cavum laryngis. Conceivably this pressure will eventually be high enough to distend the lumen of the thyrohyoid canal which is now stretched by the separation of the thyroid cartilage and the hyoid bulla. The vibrating air column will rush up the canal and into the uncollapsible hyoidean sac where the proper tones and upper tones can be intensified. An important reservation must be made, however, to what has been said: the supraglottic pressure must always be lower than the infraglottis lest the vocal cords are unable to vibrate.

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ZUSAMMENFASSUNG

Makroskopische Untersuchungen an Brüllaffen machen es wahrscheinlich dass die tiefen Töne, welche der Affe von sich gibt, durch eine wesentliche Verengerung des Vestibulum laryngis zustandekommen. Dadurch entsteht ein Überdruck der den normaler weise geschlossenen Canalis thyrohyoideus eröffnet, wodurch die schwingende Luftsäule Zutritt zur Bulla hyoidea erlangt. Die letztere wirkt als Resonator. Die Verengerung der Öffnung des Larynx kommt durch einen pneumatischen Sphinkter die pharyngolaryngeale Säcke zustande. Extralaryngeale Muskeln sind für die Aktivierung des Sphinkters verantwortlich.

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RELAPSING POLYCHONDRITIS

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Abstract Two cases of relapsing polychondritis are described, both with a high E.S.R. and without reaction to antibiotics. Both patients are men, 80 and 72 years old and both with malignant disease besides the chondral condition. One case had affection of both xternal ears with proved histopathological findings. The second case had affection of the cartilages i th larynx, trachea, nose and sterno-costal joints—and sclerokeratitis. According to the literature, the characteristic of the disease is inflammation of the cartilages, most frequently in the external ears, nose, air ways and sterno-costal joints—as well as inflammation in the eyes. Pathognomonic laboratory findings are ...ing, but a high E.S.R. is the rule. Exacerbations ... remissions are typical. The etiology is unknown—...-immunity has been discussed, as also has en...otic disorder Corticosteroids are effectiv and ...ent dramatic obstructions of the airways and destruction of cartilage.

Relapsing polychondritis is a disease characterized by inflammation and degeneration in *cartilaginous structures of the external ears*, nose, larynx, trachea and ribs. Other signs can appear in the joints and eyes. Synonyms used by various authors are polychondropathia (Jaksch-Wartenhorst, 1923), chondromalacia (Altherr 1936), chronic atrophic polychondritis (Bober & Czarniecki, 1955) diffuse perichondritis (Harwood, 1958), relapsing polychondritis (Pearson et al., 1960) atrophic polychondritis (Davies & Kelsall 1961) and panchondritis systematica (Baerthold, 1965)

The initial symptoms of the disease differ from case to case as described in the literature. One or more of the following symptoms usually appear: red and swollen external ears, joint pains, tender and swollen nasal septum, conjunctivitis, iritis, dyspnoea, cough, fever *malaise and fatigue. Studies of 56 published* cases show a slight preponderance of the female sex (31 of 56 cases) With few exceptions the patients belonged to the white race.

In 88% of the published cases the external ears are attacked earliest and most frequently With the exception of the lobulus they are, in the acute stage, red, swollen and tender and the disease is mistaken *for erysipelas*. The external meatus is frequently involved as is the cartilage of the Eustachian tube. Next, most frequently affected (82%) are the cartilages of the nose Swelling and tenderness of the nasal septum is accompanied by rhinorrhoea, erosion, crusts and epistaxis.

The disease is rare It has not been reported in Sweden, but there is reason to suppose that several cases have occurred without being diagnosed, hence the reason for presenting the following 2 cases and a review of the literature. It should be considered a serious condition, as too late a diagnosis may lead to dramatic air way obstruction (Purcelli et al 1962) and destruction of cartilage, the latter causing a cosmetically very distressing final result (Jensen, 1962) The disease has a special interest for the oto-rhino-laryngologist, as the patient consults him for the early symptoms from external ear nose or larynx.

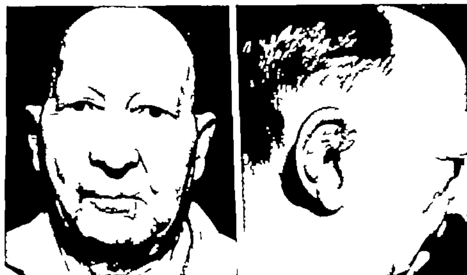


Fig. 1 Case 1 External ear before corticosteroid treatment red and swollen except for the lobulus.

Case Reports

Case 1

A man, aged 80 who in late April 1966 got a sudden pain in his left external ear When examined in May one external ear except for the lobulus, was red and swollen (Fig. 1) The patient had a normal body temperature, E.S.R. 123 mm/hr leucocytes 4 200/mm³ and normal urine on laboratory examination. The electrophoresis showed total protein 8.8 g% albumin 4.3 alfa-I-globulin 0 4 alfa-II-globulin 0 9 beta-I 0 6, beta-II 0.5 and gammaglobulin 2 1 g% i.e., mainly a rise of gammaglobulin. According to the report, the electrophoresis was described as indicative of a chronic process with some activity Repeated electrophoresis gave the same answer On a supposed diagnosis of erysipelas, large doses of V-penicillin and sulfa were given. When after 5 days of this treatment no result was seen, tetracyclines were administered. Still no improvement occurred and the other ear now started to show the same symptoms. The E.S R. remained over 100 mm/hr but body temperature was normal. X-ray investigations of the nasal sinuses and thorax were normal, those of the lungs showed no active pathology Ophthalmic investigation was negative. No bacteria appeared on culture, AST<50, ASTA<2. Bone marrow was normal A biopsy from the external ear showed an abundance of leucocytes in the chorium and oedematous parts with inflammatory cells reaching the cartilage, which was degenerated—histologically a picture of non-specific inflammatory reaction with marked degeneration of the cartilage, i.e. a relapsing polychondritis (Fig. 2) Corticosteroids (prednisolone, ACO[®]) were given, and the continuous daily dose after the initial high doses was 2.5 mg × 3 The E.S.R. diminished, and within a week the pathological appearance of the external ears regressed. As the E S R. in August had risen to 73 mm/hr the dose was raised to 5 mg × 2, in December to 5 mg × 3 following which the E.S R. diminished to 42 mm/hr The external ears had by then become completely normal. No more cartilages had been attacked, nor had the eyes. Icterus with laboratory findings typical of stasis now appeared. Exploratory laparotomy showed pancreatic cancer The corticosteroid medication continued, but the patient died from his pancreatic disease in January 1967 in another clinic without any further symptoms having appeared in the other cartilages in his body

Fig. 2. Case 1. Photomicrograph of biopsy from external ear. Abundance of leucocytes in chorium and oedematou the cartilag

nflammatory cells reaching generated.



Fig. 3 Case 2. Laryngoscopic picture. Thick vocal cords leave narrow rima glottidis even at abduction. Note the insufficient abduction

Case 2

A man, aged 72, still alive After 2 weeks of slight pharyngeal pains with fever and symptoms of laryngeal stridor for 2 days, the patient was first seen in October 1966 The E.S.R. was 67 mm/hr The general practitioner had given inhalations, antiinflammatory drugs and tetracyclines. The examination revealed oedematous vocal cords with only a few mm space left dorsal in the larynx. Even at rest, the patient had dyspnoea. Direct laryngoscopy showed oedema in the larynx and upper part of trachea. The patient had pains from the cervical spine and the sterno-costal joints His right eye showed pericorneal injection and oedema in the marginal parts of the cornea with demonstrable ulcerations, as well as precipitates inside the eye As the oedema of the larynx was obstructing the airway corticosteroids were given parenterally After this treatment the oedema of the larynx diminished and the pains from the cervical spine and sterno-costal joints disappeared. Biopsies from the larynx was not taken as disasters after even smaller surgical procedures in the larynx are described in literature (Kaye & Sones 1964 Purcelli et al., 1962) Biopsies were taken from the sterno-costal joints where the patient had felt pains and tenderness and from the external ear and nasal septum where he had no symptoms. The patient had by then been treated with corticosteroids and only laryngeal symptoms remained No histopathological signs were seen. Withdrawal of corticosteroids was tried, whereupon the symptoms of laryngeal oedema with stridor and pains in the neck and thorax returned, on one occasion accompanied by a swollen and painful nose Since then he has not been able to do without the corticosteroids until April 1970 His current dose was 5 mg prednisolone per day Dysuria has led to surgical intervention with electroresection of the prostate gland Bi-



Fig. 4 Case 2. Tomogram of larynx after 2 years of corticosteroid therapy The vocal cords swollen. The distance between the vocal cord is even at respiration not more than 5 mm in the frontal plane.

opsy showed cancer of the prostate, for which oestrogens (estradurin®) are given monthly In addition, radiation therapy was given for a small basal cell carcinoma on the cheek. The larynx is still narrow with thick vocal cords not fully mobile seen at direct laryngoscopy and tomogram (Figs 3 4).

DISCUSSION

The 2 cases presented above show some of the typical signs of relapsing polychondritis. Characteristic of the disease is the involvement of more than one chondral structure. The course is generally chronic, and may be relapsing, as was noted in the second case There are no typical laboratory findings, (Yamazaki et al. 1966) but in 91% of the cases described in the literature the E.S.R. is very high, as in our 2 cases. Rheumaserology is negative. Although there is no pathognomonic histologic finding, (Kaye & Sones 1964) there is remarkable consistency of the microscopic picture. The chondrocytes lose their cytoplasm until eventually only nuclear remnants are found in involved areas Fibrous connective tissue grows inward the edge of the cartilaginous plate to re the destroyed and fragmented areas of ge. At the interface of cartilage and con ctive tissue an inflammatory infiltration with plasma cells and lymphocytes is seen during periods of acute inflammation. The inflammatory reaction regresses during periods of remission. Eventually new bone formation may occur (Goldwater 1963 Pearson et al. 1960 Purcelli et al., 1962 Strobel & Seifert, 1960) In the second case we found complications in the eye, which in the literature are described in 60% of the cases (Dolan et al 1966) No connection with any other disease has been proved by any author Coincidence with many systemic diseases has been found—but not significantly for any particular one (Rosen et al., 1969) Both our patients had malignant disease and that suggests a new point of view Is relapsing polychondritis a paramalign manifestation? Coincidence with malignity has not been noted before. In the literature some symptoms of the disease are mentioned which are not represented in our 2 cases. Among these are involvement of the temporomandibular and extremity joints (Kaye & Sones, 1964) together with their cartilages. Furthermore involvement of the cartilages of the respiratory system may be even more prominent, including the epiglottis, trachea and bronchi (Daly 1966 Kaye & Sones, 1964 Purcelli et al 1962 Thould et al., 1965)

In untreated cases, after a relapsing course, deformity occurs with atrophic hanging external ears saddle nose, deformed joints with destruction of the articular cartilage, collapse of the trachea with obstruction of the airway chronic bronchitis and respiratory insufficiency Amaurosis has also been reported (Barth & Berson 1968)

The similarity of the disease to other conditions makes differential diagnosis a problem. The articular involvement resembles certain forms of rheumatoid arthritis. Small as well as large extremity joints, and the intervertebral joints, may be involved. The course of the disease is not the same as that of rheumatoid arthritis, and affection of the sternocostal joints, typical in polychondritis, is very rare in rheumatoid arthritis. X ray examination of the joints may in polychondritis, show reduction of the articular space caused by loss of articular cartilage, but does not show erosions as in rheumatoid arthritis (Barth & Berson, 1968). Histologically the difference is obvious, as the findings in rheumatoid arthritis are typical of chronic inflammation, hypertrophy of the synovia with the subchondral bone being attacked before the cartilage. In polychondritis, the cartilage is attacked first, and the bone second. Rheumaserology is negative in relapsing polychondritis Other diseases to differentiate between are erysipelas, Wegener's granulomatosis and the syndromes of Reiter and Sjögren (Goldwater 1963 Kaye & Sones, 1964 Yamazaki et al., 1966)

The etiology is unknown. The condition may be auto-immune in nature (Dolan et al 1966

Harwood, 1958 Jordans et al., 1966) No bacteria, fungi or specific toxins have been found in the blood of cases with atrophic polychondritis. There is no evidence of a hereditary factor The resemblance to rheumatoid arthritis, and the success of corticosteroid therapy suggest the disease may belong to the group of autoimmune conditions involving connective tissue, although complement binding antibodies have not been demonstrated. Some authors have suggested the origin to be metabolic or enzymatic defects (Dingle, 1962) Experimentally chondrolysis in rabbit ears has been produced by papain given intravenously this effect being caused by the enzymatic influence on chondromucoprotein.

The 2 cases described above support the theory that relapsing polychondritis may be a paramalign manifestation.

The treatment of the disease is partly surgical, partly pharmacological. Salicylate in doses of 2–5 gm per day has been shown to have some effect on some patients. (Kaye & Sones 1964 Thould et al., 1965) The drugs of choice are corticosteroids (Davies & Kelsall, 1961 Goldwater 1963 Johnson, 1963 Kaye & Sones, 1964 Pearson et al 1960) and most authors have used an initial dose of 30 mg Prednisolone, ACO[R] the first day with successively reduced doses on the following days. During remissions, the patients have been without treatment. Brochoscopies, with bronchial toilette and tracheotomies, have in some cases been necessary (Bober & Czarniecki, 1955 Daly 1966 Harders, 1954 Strobel & Seifert, 1960) Some patients have become permanently cannulated

ZUSAMMENFASSUNG

Zwei Fälle von chronischen atrophischen Polychondritiden sind beschrieben, die beide durch hohe Senkungsreaktion und fehlende Antwort auf Antibiotika charakterisiert sind. Corticosteroide waren indessen wirkungsvoll. Beide Patienten, Männer im Alter von 80 und 72 Jahren, hatten neben ihren Knorpelaffektionen auch maligne Krankheiten. Der eine hatte beidseitiger Aussenohraffektion mit histopathologischen Befunden, der andere hatte eine Knorpelaffektion die die Knorpel des Kehlkopfes, der Luftröhre der Nase und der Sternocostalgelenke einbezogen haben. Ausserdem lag bei ihm ein Skleroleratitis vor Die Literatur beschreibt diese Krankheit als eine Knorpelentzündung, die am häufigsten an den Knorpeln des Aussenohres, der Luftwege und der Sternocostalgelenke gebunden ist. Die Augenaffektion ist ein weiteres Charakteristikum. Ausser einer erhöhten Senkungsreaktion findet man keine typischen Laborwerte. Typisch sind Exacerbationen und Remissionen. Die Ätiologi ist unbekannt, Man hat sowohl eine enzymatische Immungenese wie eine Autoimmungenese diskutiert. Die Behandlung mit Corticosteroiden ist wirkungsvoll und erhindert weitgehend den Knorpelabbau und den Verschluss der Luftwege.

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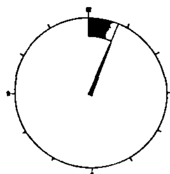
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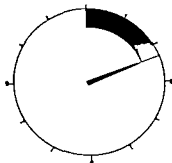
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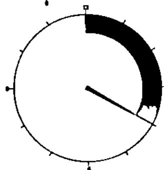
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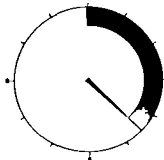


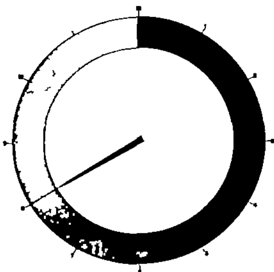
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